

# FIGURE 1. Pro104.D116.1 MAb Binds to 293F Cells Transiently Transfected with Pro104

Fig. 1A

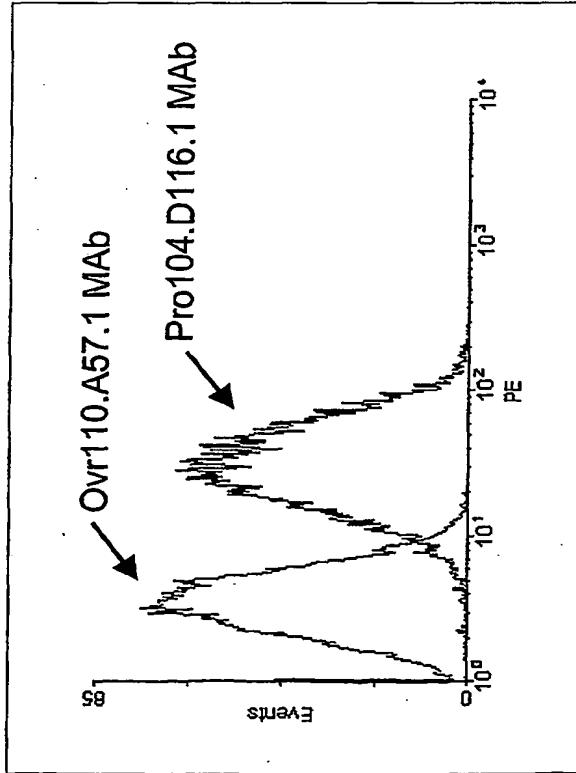
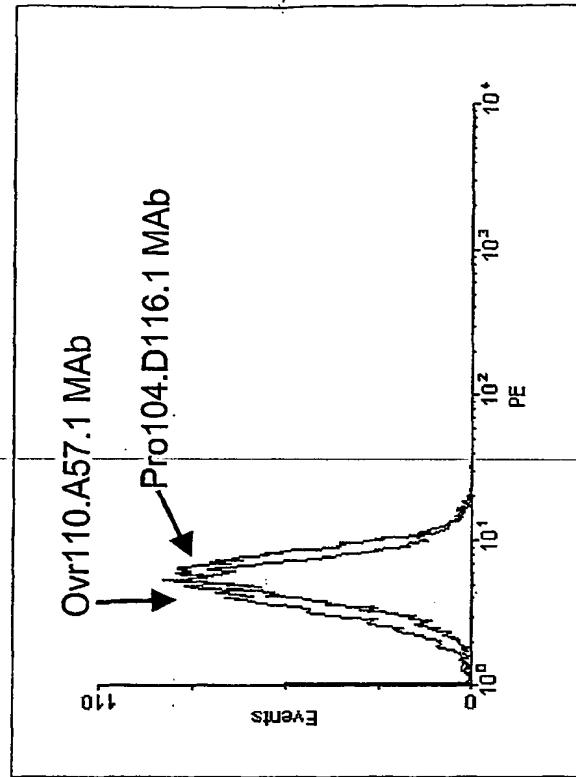


Fig. 1B



**Pro104-Transfected 293F Cells**

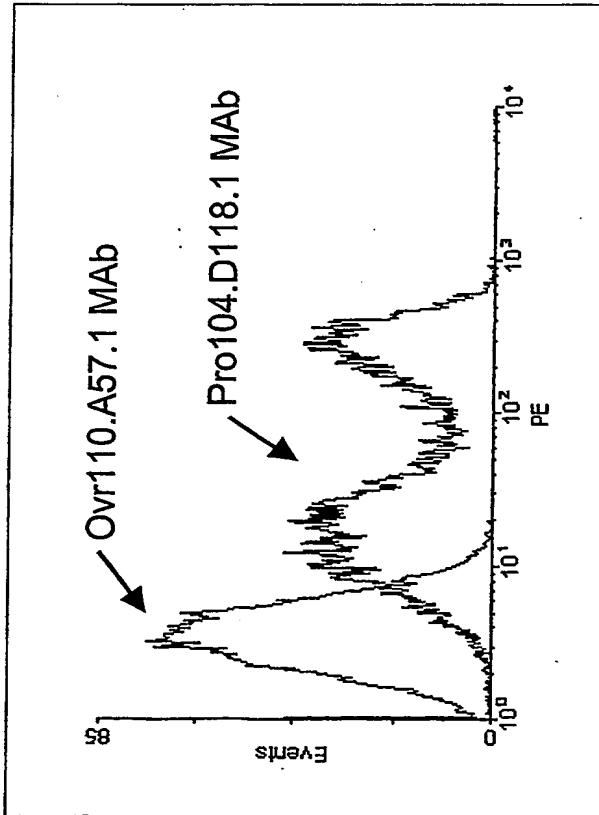
**Untransfected 293F Cells**

No Fill: Pro104.D116.1 MAb  
Shaded: Ovr110.A57.1 Control MAb

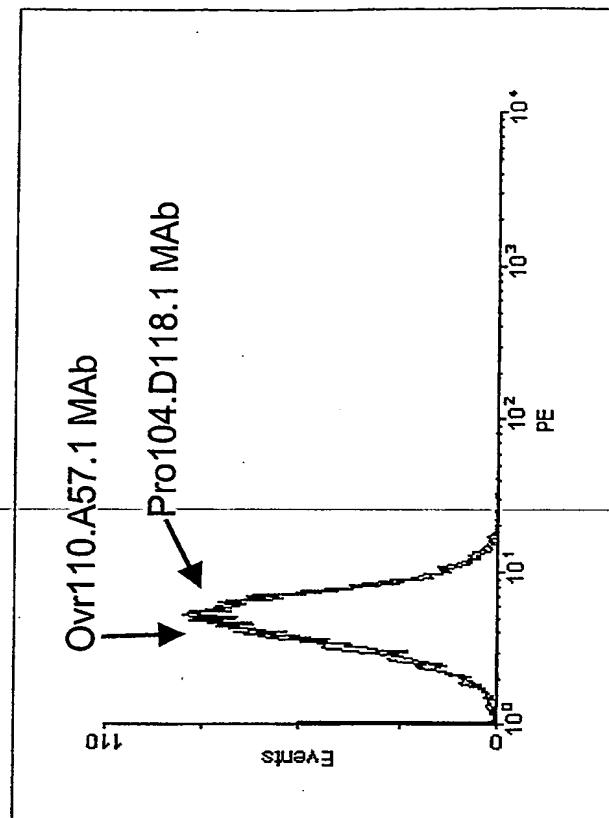
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**FIGURE 2. Pro104.D118.1 MAb Binds to 293F Cells  
Transiently Transfected with Pro104**

**Fig. 2A**



**Fig. 2B**



**Pro104-Transfected 293F Cells**

**Untransfected 293F Cells**

No Fill: Pro104.D118.1 MAb

Shaded: Ovr110.A57.1 Control MAb

**FIGURE 3. Pro104.C19.1 binds to live HeLa  
Cancer Cells Expressing Pro104**

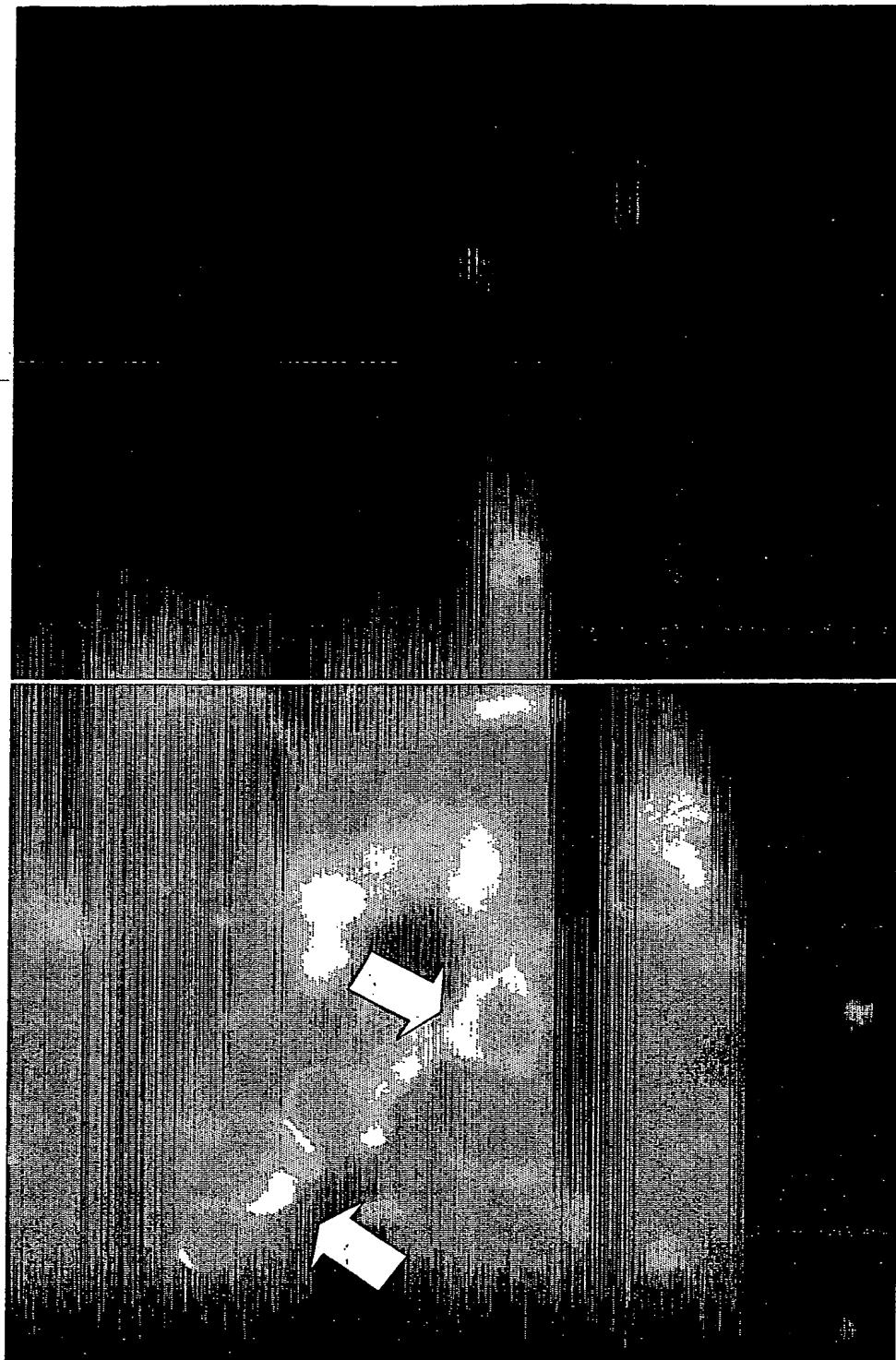
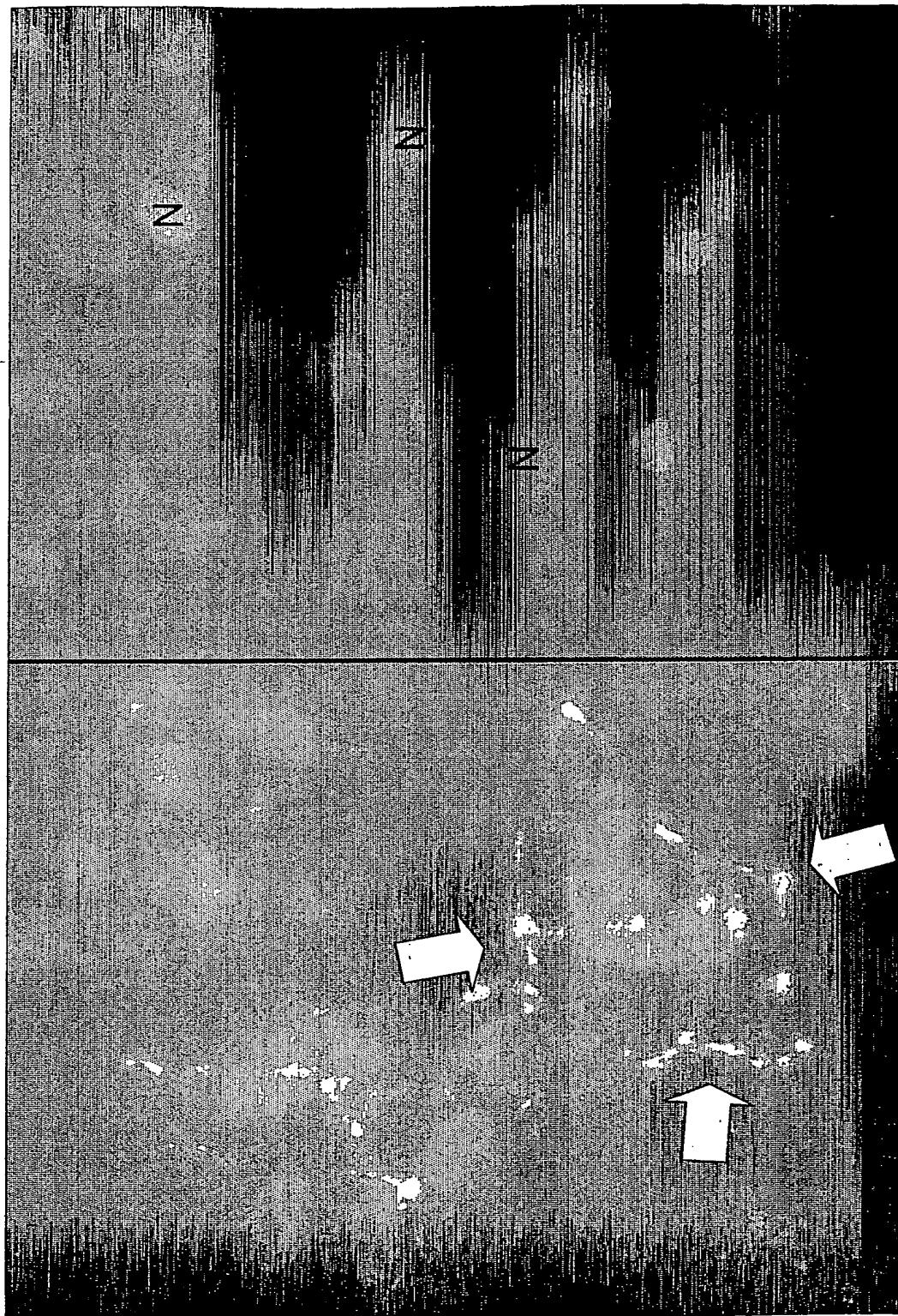


Fig. 3A. HeLa Cells

Fig. 3B. SKOV-3 cells

N= Nuclei

**FIGURE 4. Cy3-Pro104.C25.1 binds to live HeLa Cancer Cells Expressing Pro104**

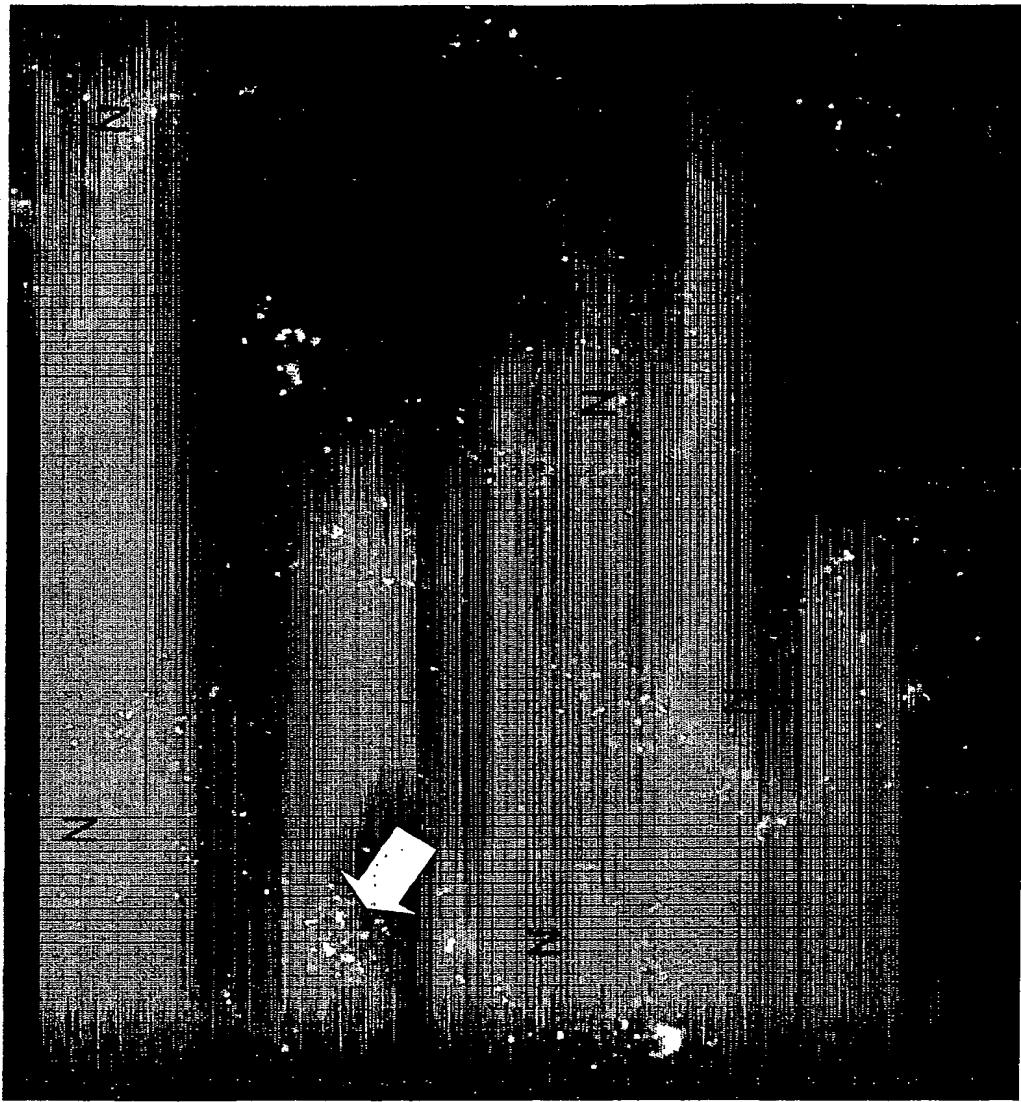


**Fig. 4A. HeLa Cells**

**Fig. 4B. SKOV-3 cells**

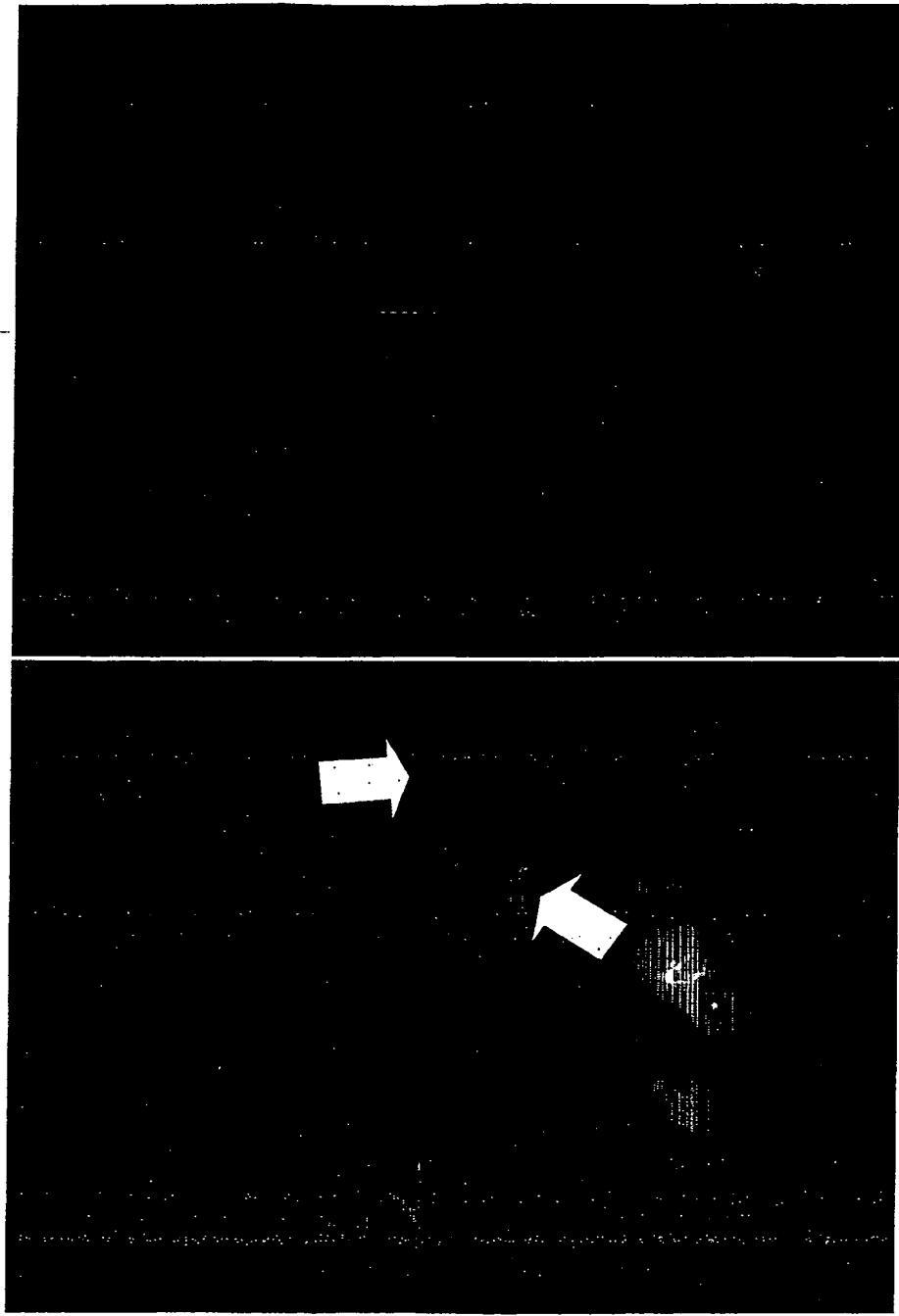
N= Nuclei

**FIGURE 5. Cy3-Pro104.C25.1 binds to and is Internalized in live HeLa Cancer Cells Expressing Pro104**



N= Nuclei

**FIGURE 6. Cy3-Pro104.C19.1 binds to and is Internalized in Pancreatic Cancer Cells Expressing Pro104**



**Fig. 6A. MIA PaCa-2 Cells**

**Fig. 6B. HCT-116 cells**

N= Nuclei

**FIGURE 7. Cy3-Pro104.C55.1 binds to and is Internalized in Pancreatic Cancer Cells Expressing Pro104**

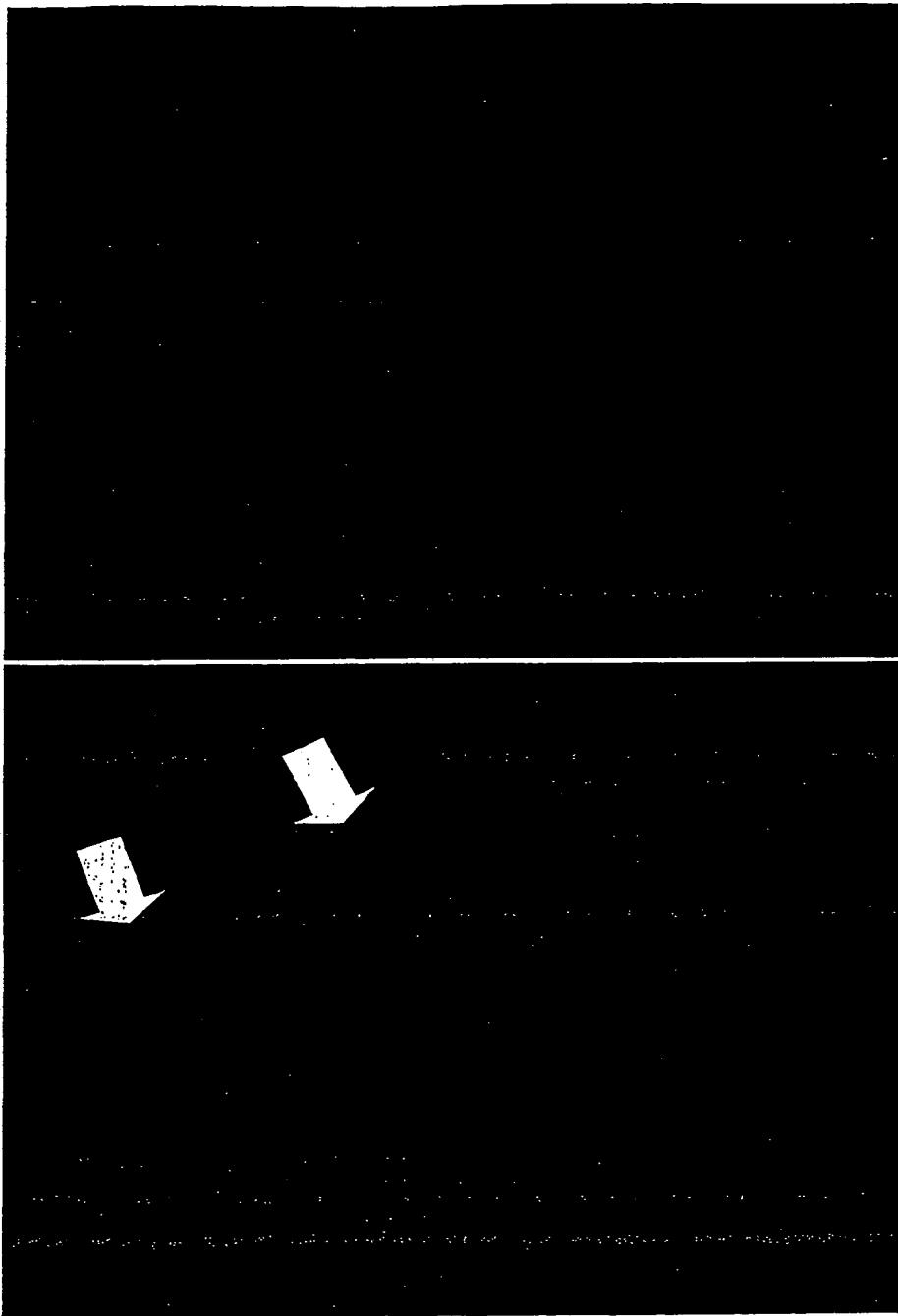


Fig. 7A. MIA PaCa-2 Cells

Fig. 7B. HCT-116 cells

N= Nuclei

# FIGURE 8. Pro104.C25.1 binds to Pro104 on Cancer Cells in Ovarian Tumors



Fig. 8A ovarian cancer 1

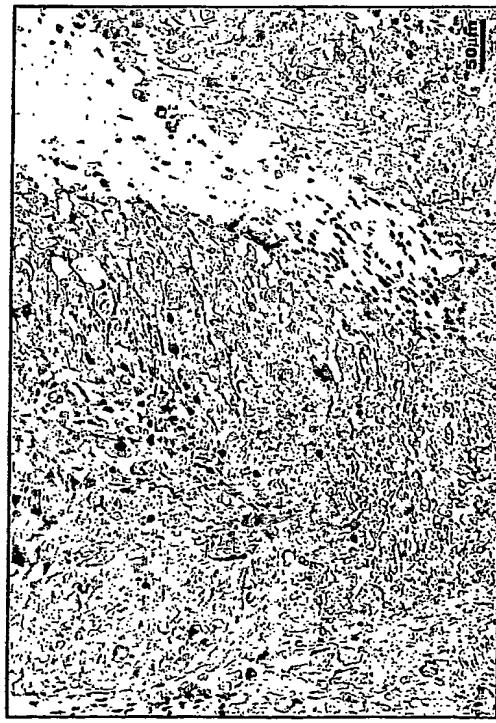


Fig. 8B normal ovary 1

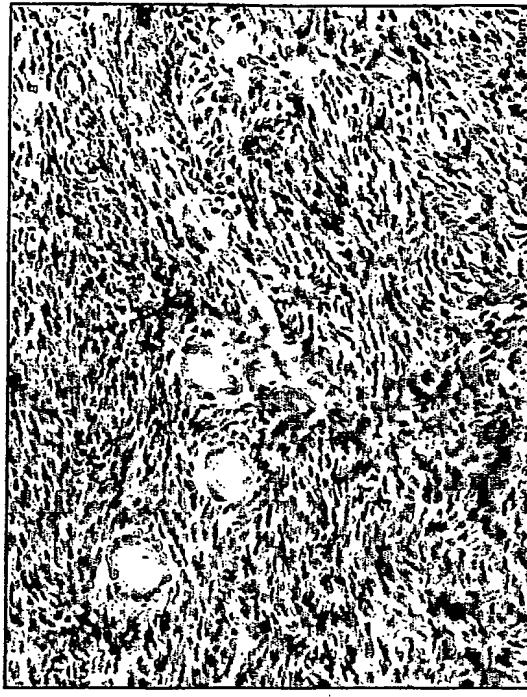
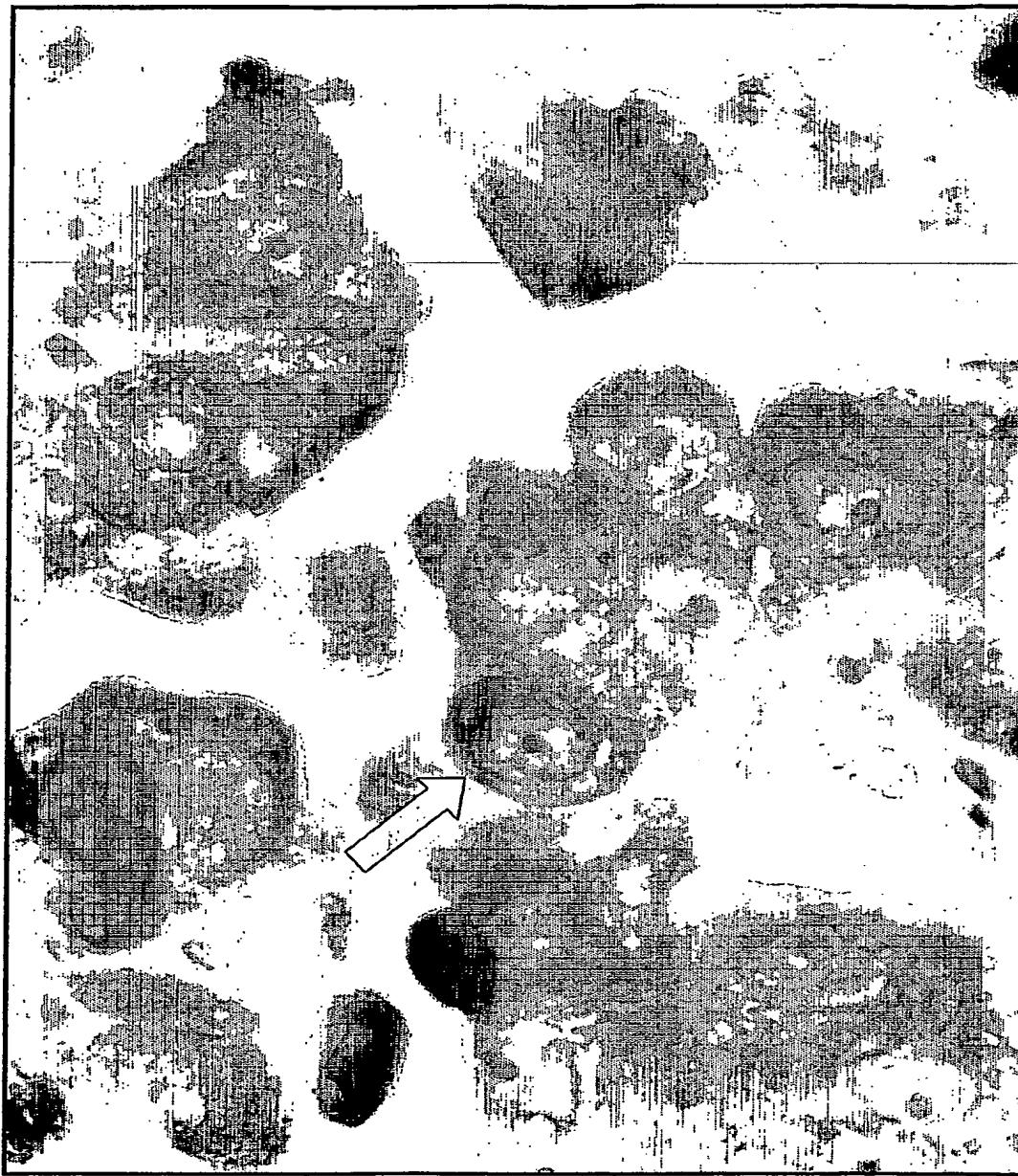


Fig. 8C ovarian cancer 2  
Fig. 8D normal ovary 2

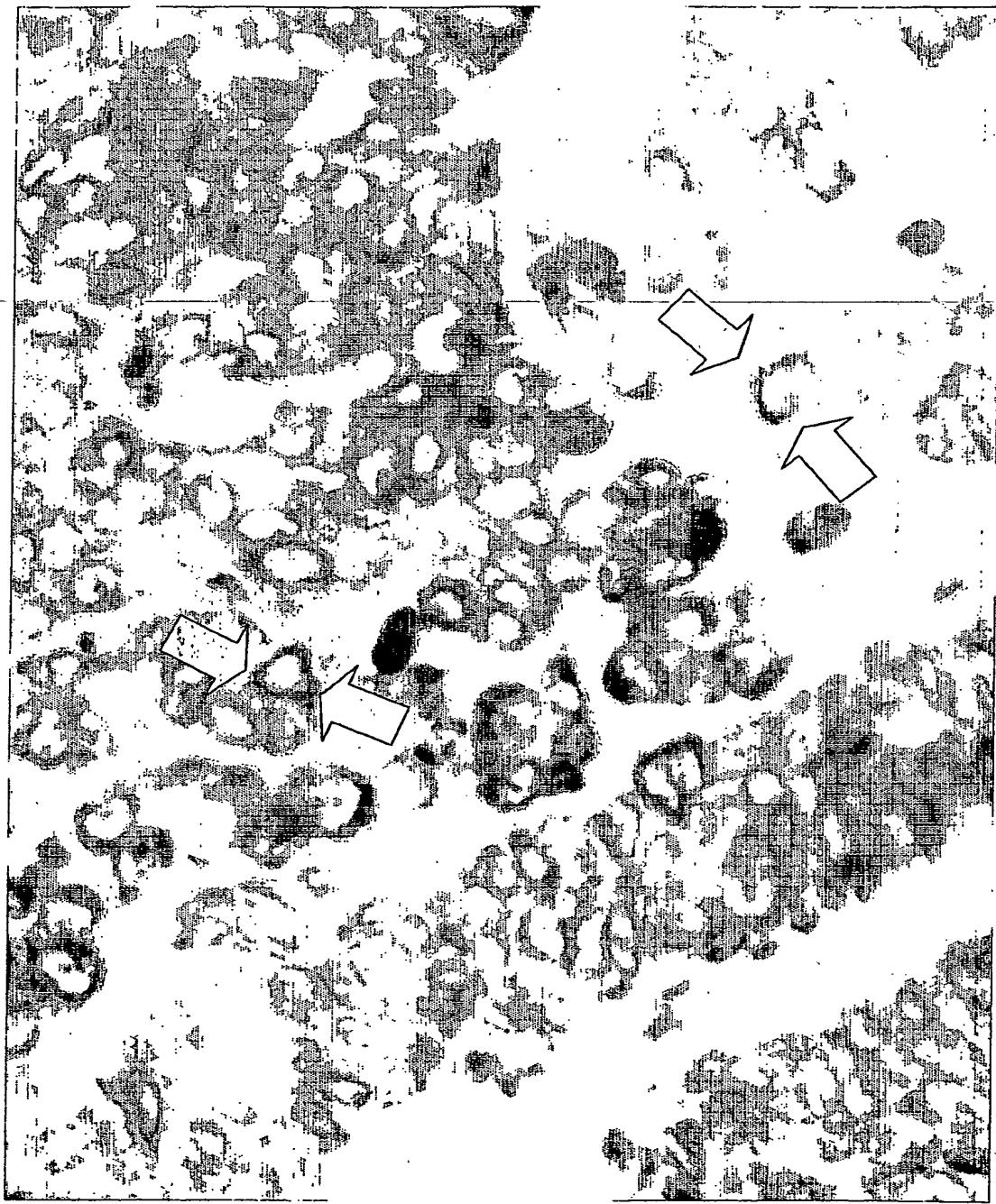
**FIGURE 9. Pro104.C25.1 binds to Pro104 on the Cell Membrane of Ovarian Cancer Cells**



**FIGURE 10. Pro104.D9 binds to Pro104 on the Cell Membrane of Ovarian Cancer Cells**



**FIGURE 11. Pro104.D133 binds to Pro104 on the Cell Membrane of Serous Ovarian Cancer Cells**



## FIGURE 12. Pro104.C25.1 binds to Pro104 on Cancer Cells in Pancreatic Tumors



Fig. 12A Pancreatic cancer 1

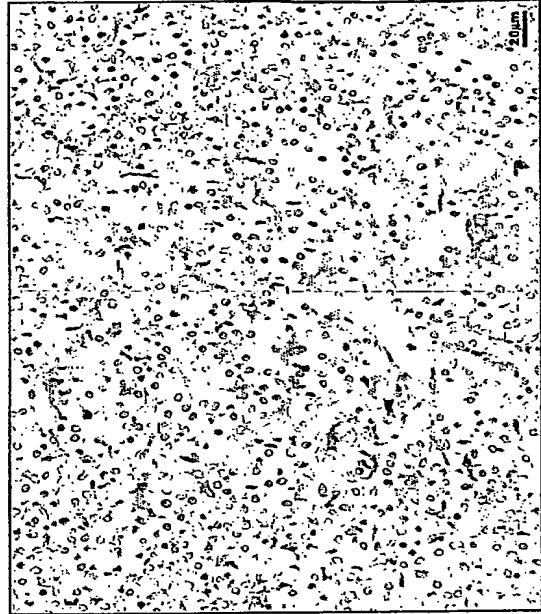


Fig. 12B Normal pancreas 1

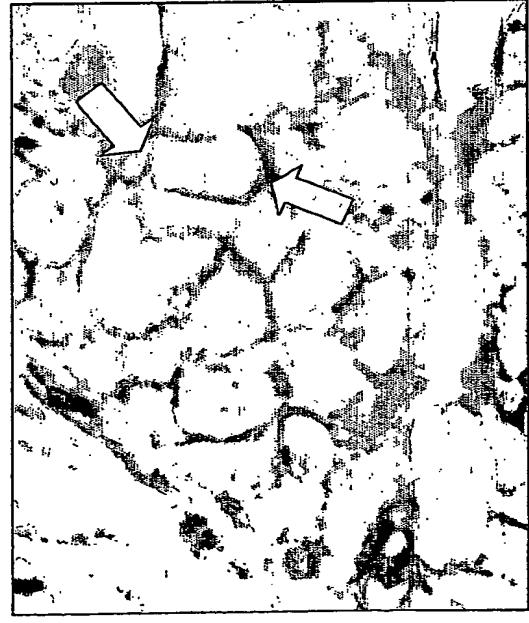


Fig. 12C Pancreatic cancer 2

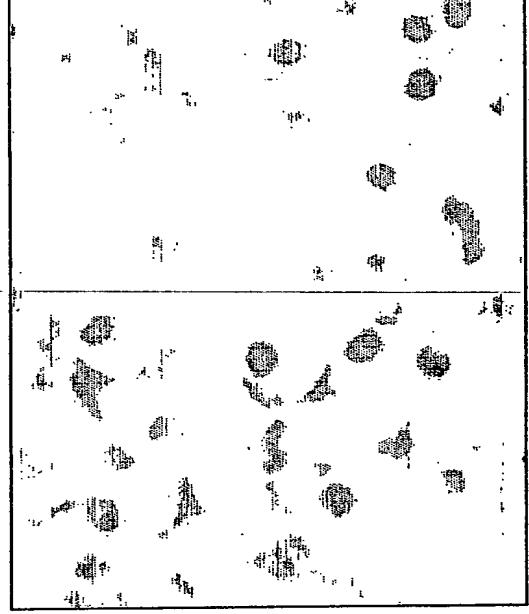
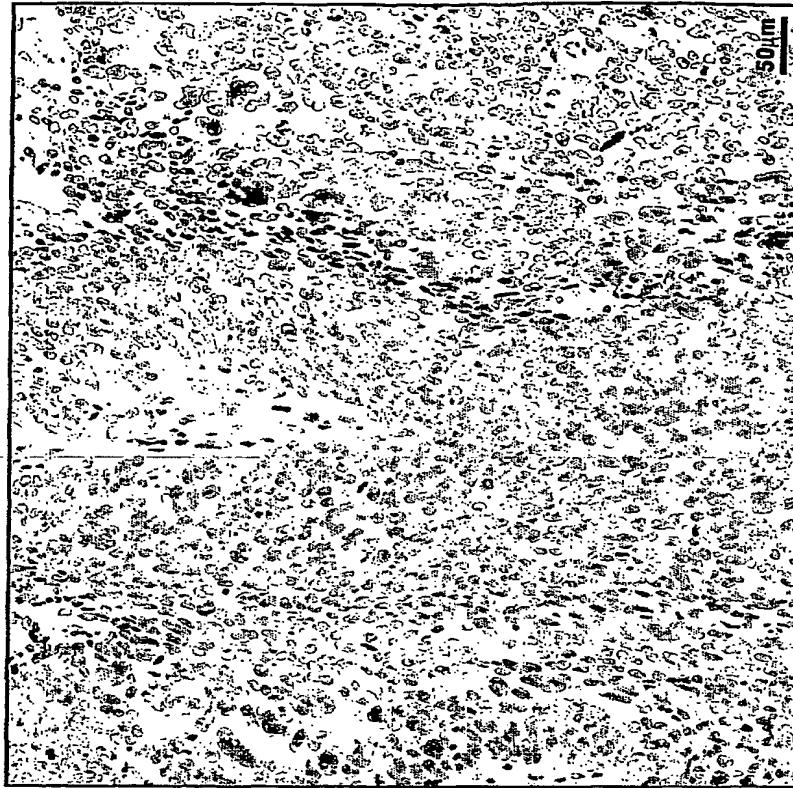


Fig. 12D Normal pancreas 2

**FIGURE 13. Controls Demonstrating Pro104 MAb Immunolabeling Specificity**

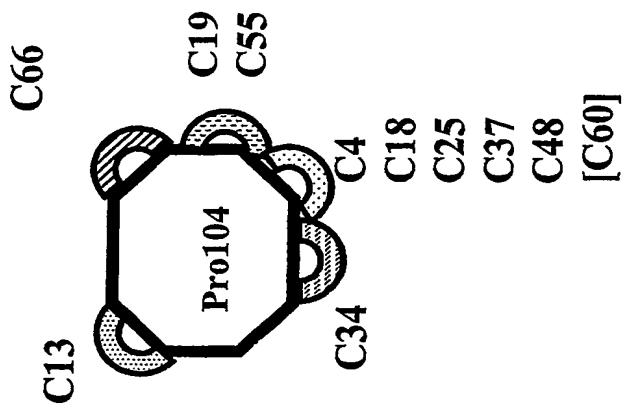


**Fig. 13A Mouse IgG**



**Fig. 13B Absorption with Pro104 Antigen**

**Figure 14: Epitope Map of Pro104 MAbs**



**Pairs tested on Training Serum and Cell Line Panel:**  
**C4/ C13; C48/ C13; C13/ C18; C19/ C48; C55/ C34; C66/ C18; C19/C25; C55/C25**

**FIGURE 15**

**Western Blot Showing Detection of Pro104 Protein in mRNA+ Cell Lines and Ovarian Tumor Tissue (T) but not Normal Adjacent Tissue (N)**

Fig. 15A

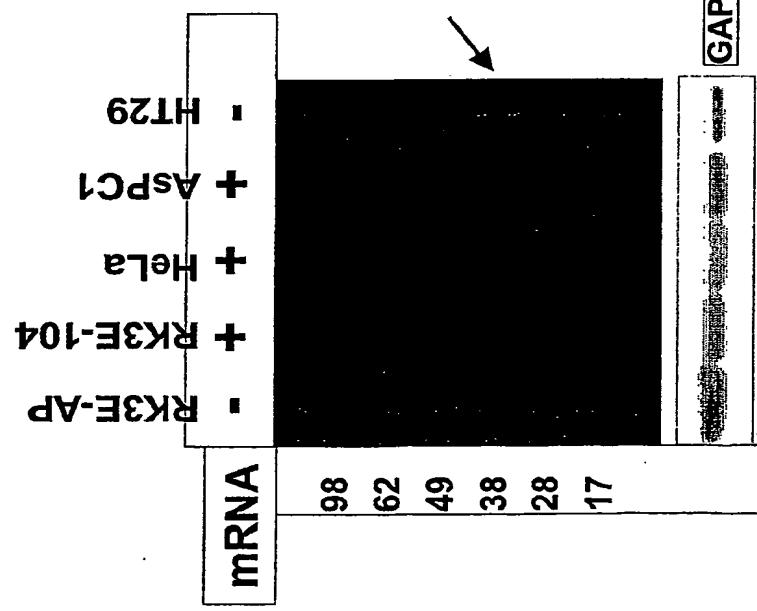
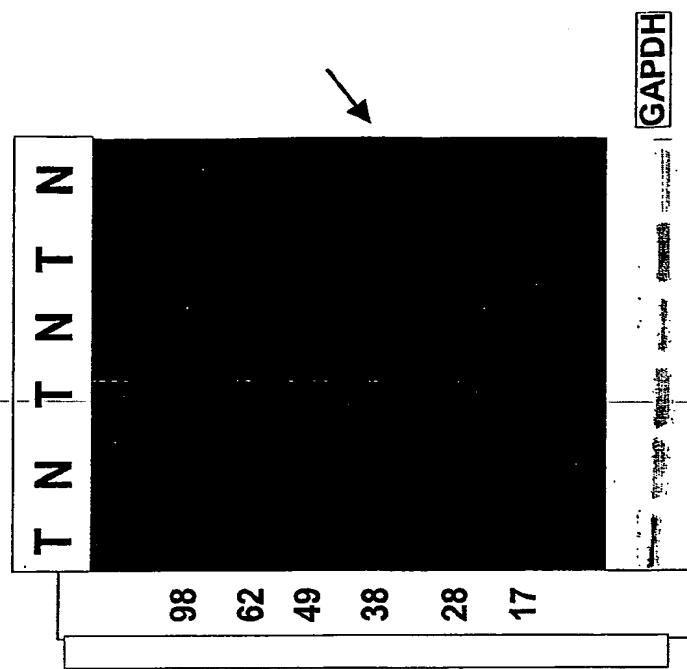


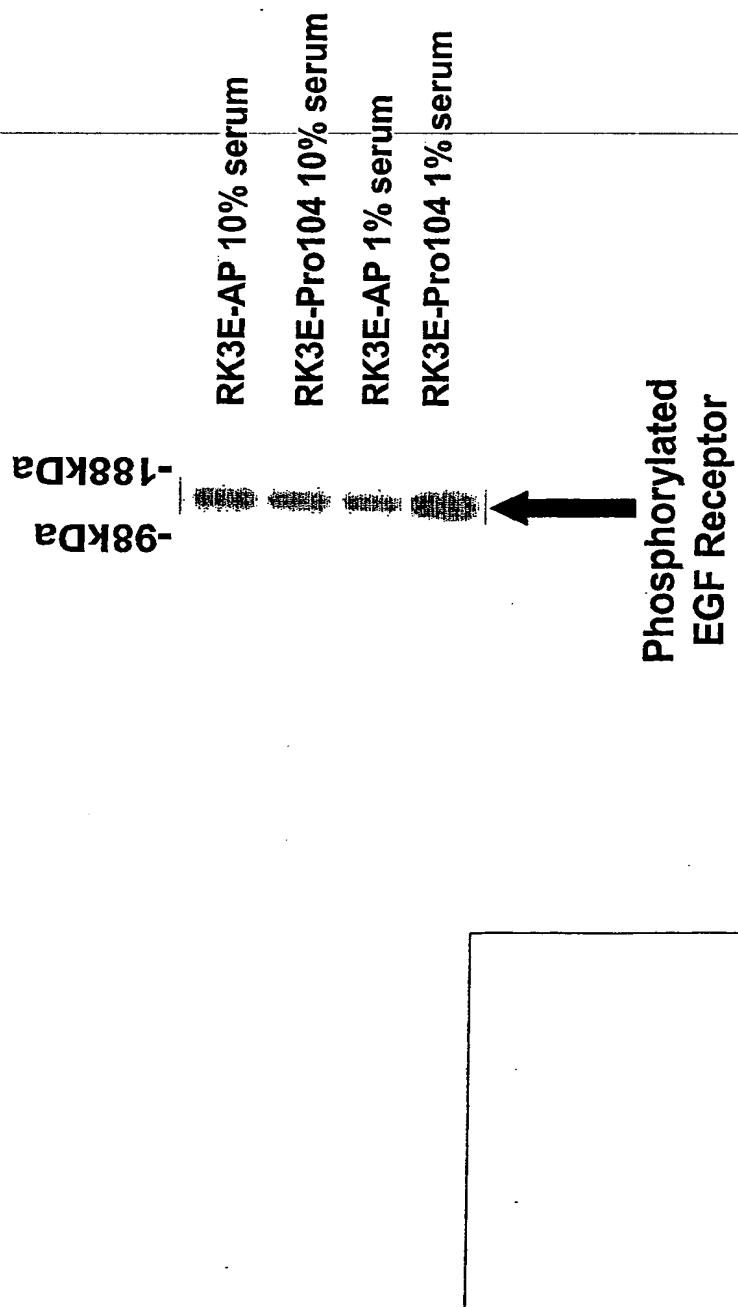
Fig. 15B



Arrow indicates location of Pro104 band

## FIGURE 16

### Overexpression of Pro<sup>104</sup> Leads to Phosphorylation of EGF Receptor



Immunoblot With Antibody Against Phosphorylated EGF Receptor

# FIGURE 17

## Pro104 Protein is Glycosylated and GPI-Linked

Fig. 16A

Native Pro104 is de-glycosylated in HeLa cell lines and ovarian tumors when treated with PNGaseF

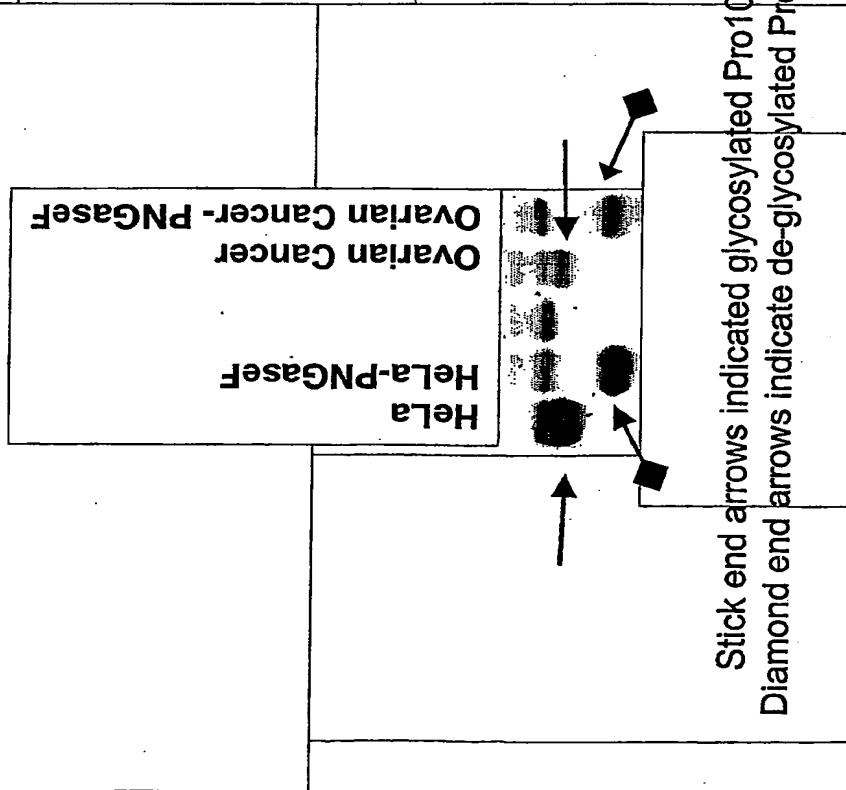
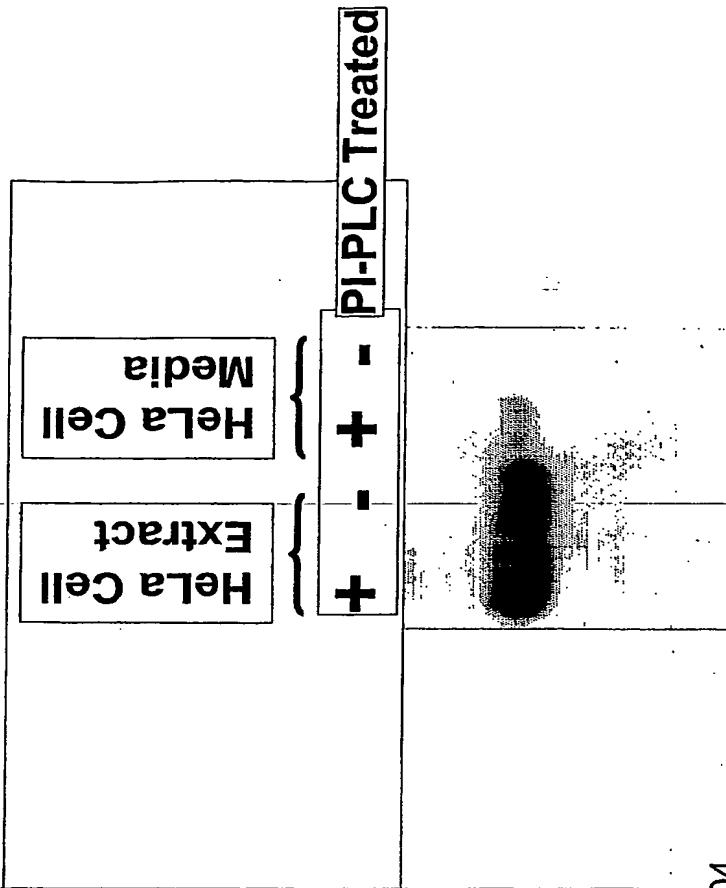


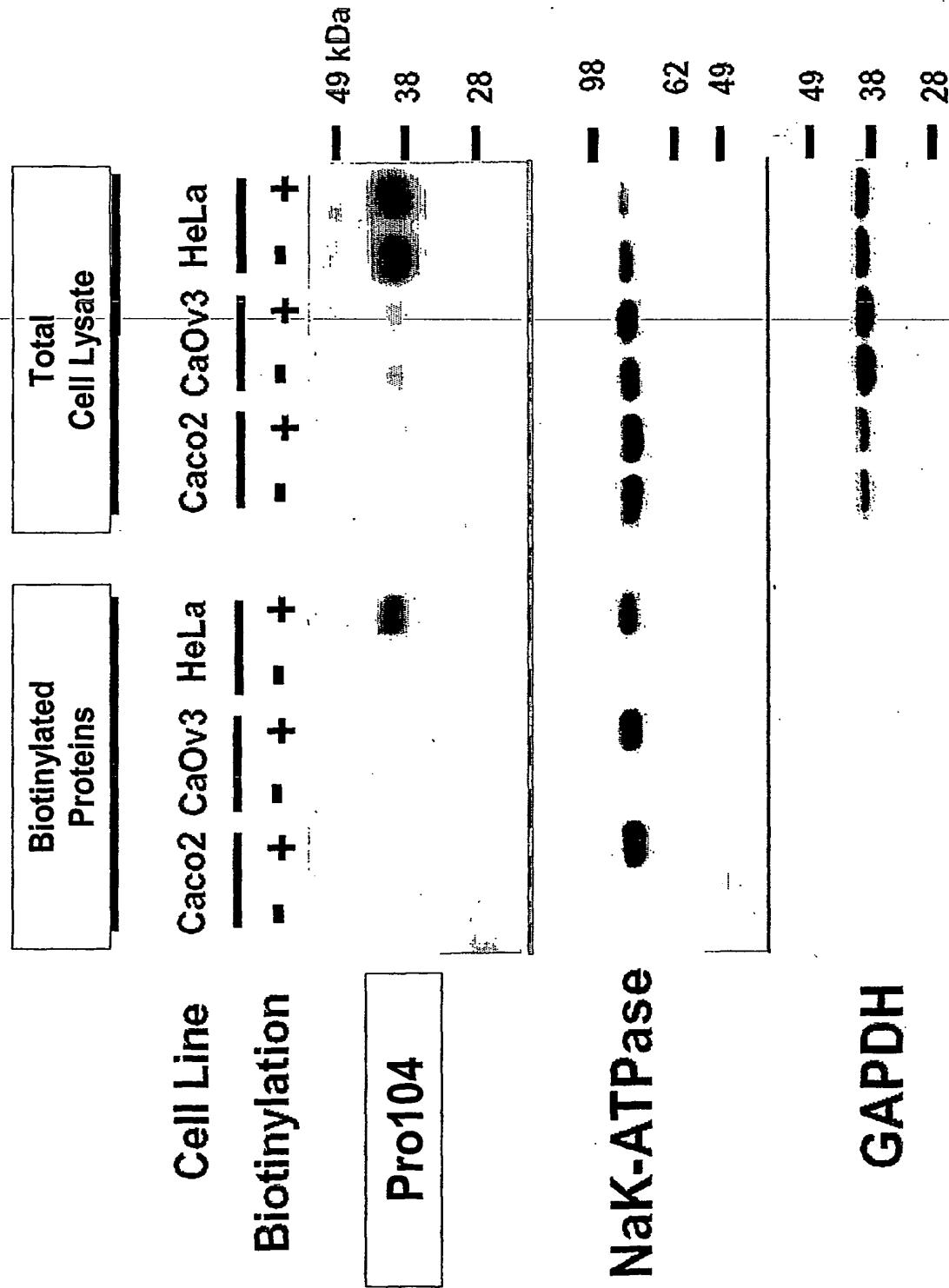
Fig. 16B

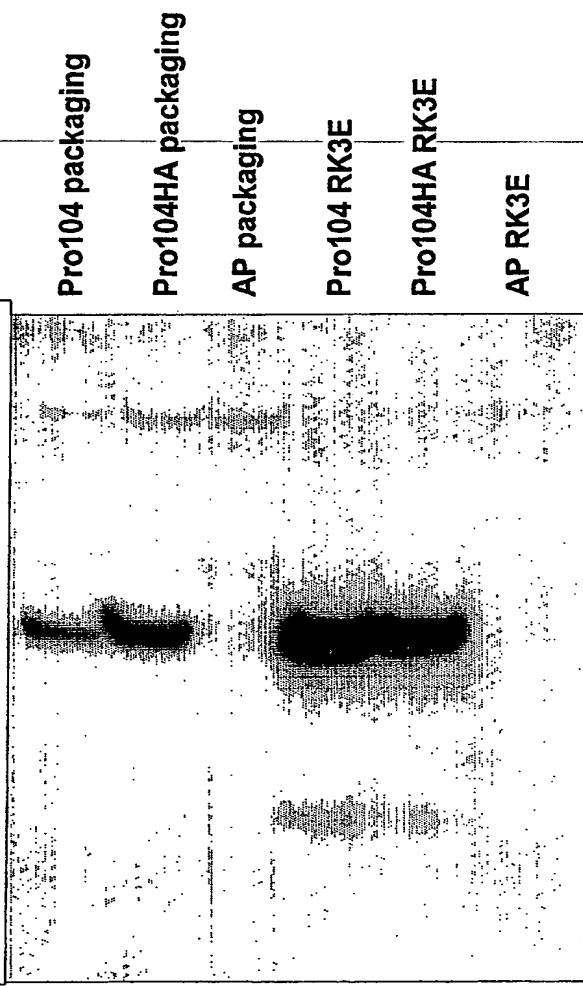
Native Pro104 shed into the media when treated with PI-PLC



## FIGURE 18

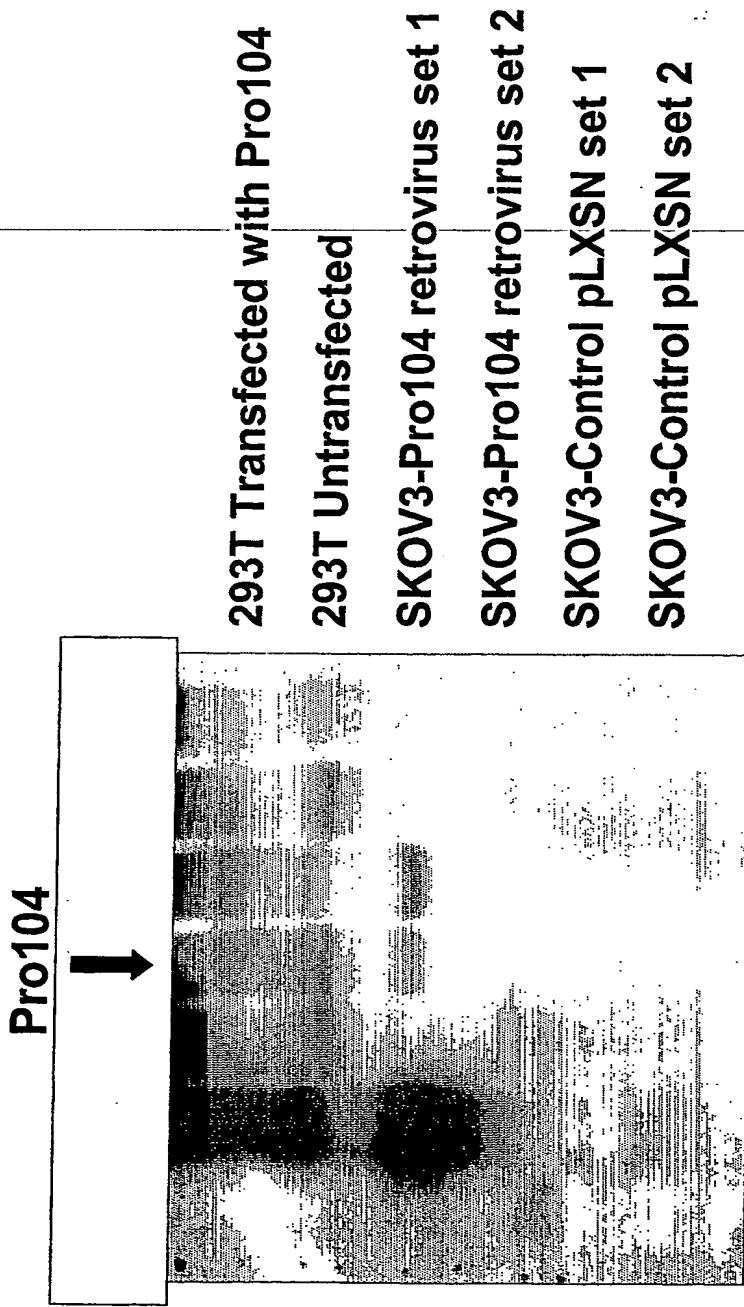
### Surface Biotinylation of Native Pro104 in Cell Lines



**FIGURE 19****Retroviral-Mediated Overexpression of Pro104 Protein in RK3E Cells**Pro104  
↓**Blot Anti-Pro-104****Western Immunoblot demonstrating Pro104 protein expression in retroviral packaging cell lines and virus infected RK3E cells**

## FIGURE 20

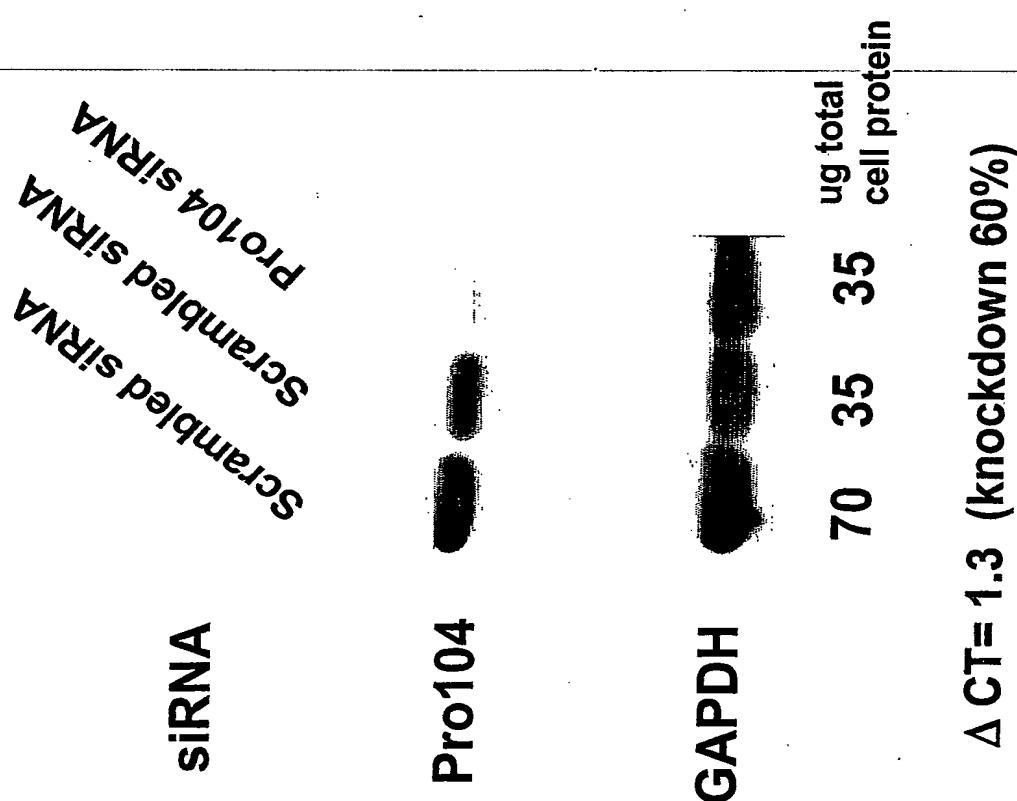
### Retroviral-Mediated Overexpression of Pro104 Protein in SKOV3 Cells



Western Immunoblot demonstrating Pro104 protein expression in retroviral packaging cell lines and virus infected SKOV3 cells

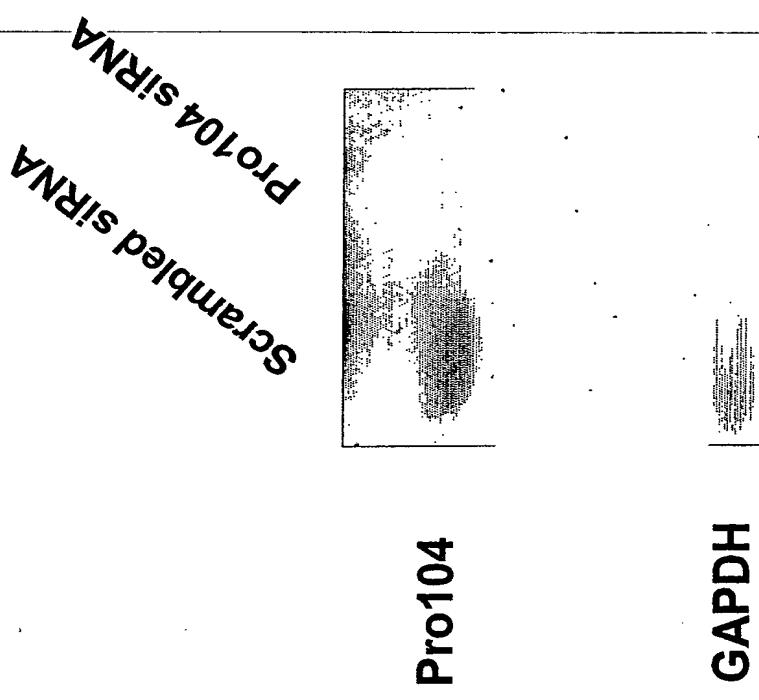
## FIGURE 21

### siRNA Mediates Specific Down-Regulation of Pro104 Protein in HeLa Cells



## FIGURE 22

### siRNA Mediates Down-Regulation of Pro104 Protein in CaOV3 Cells



$$\Delta CT = 1.2 \text{ (knockdown 55\%)}$$

# FIGURE 23

## Pro104 siRNA Specifically Knockdown Pro104 mRNA in CaOV3 Cells

Fig. 23A

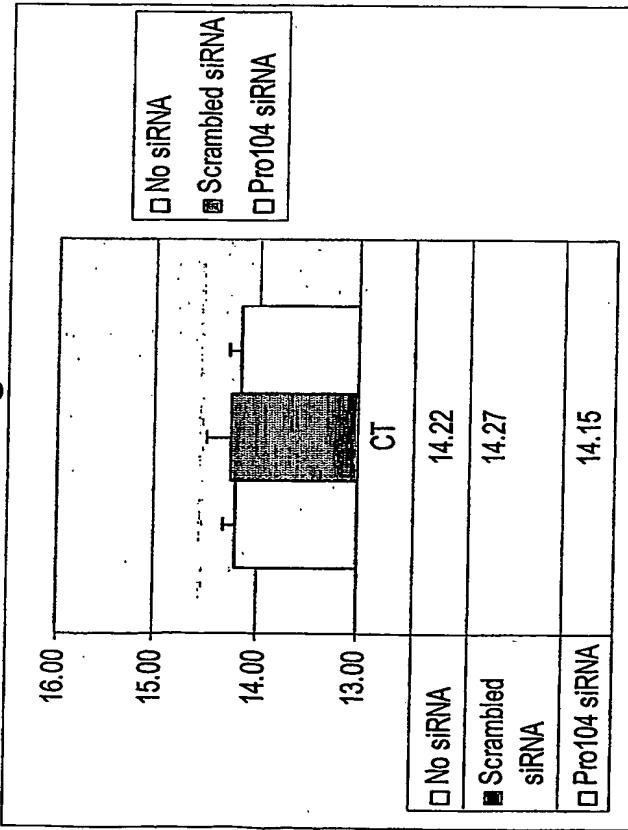
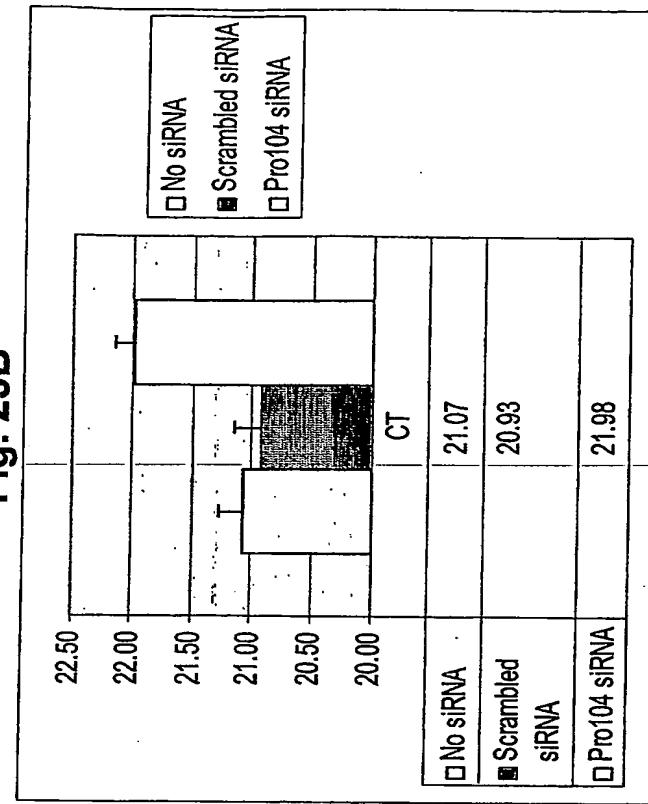


Fig. 23B



**GAPDH Q-PCR Primers**

**Pro104 Q-PCR Primers**

$\Delta CT = 1$  for Pro104 (Knockdown 50%)

## FIGURE 24

### Pro104 siRNA Specifically Knockdown Pro104 mRNA in HeLa Cells

Fig. 24A

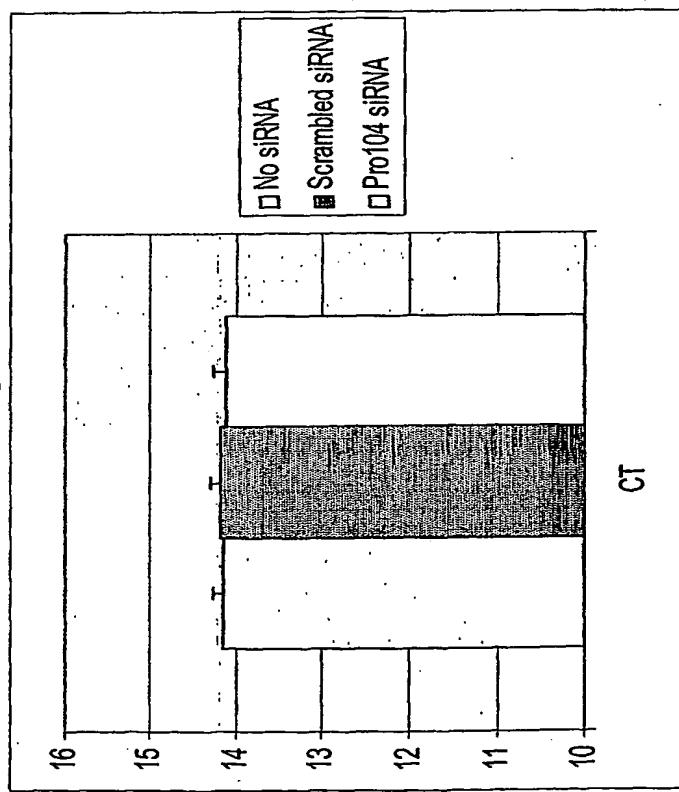
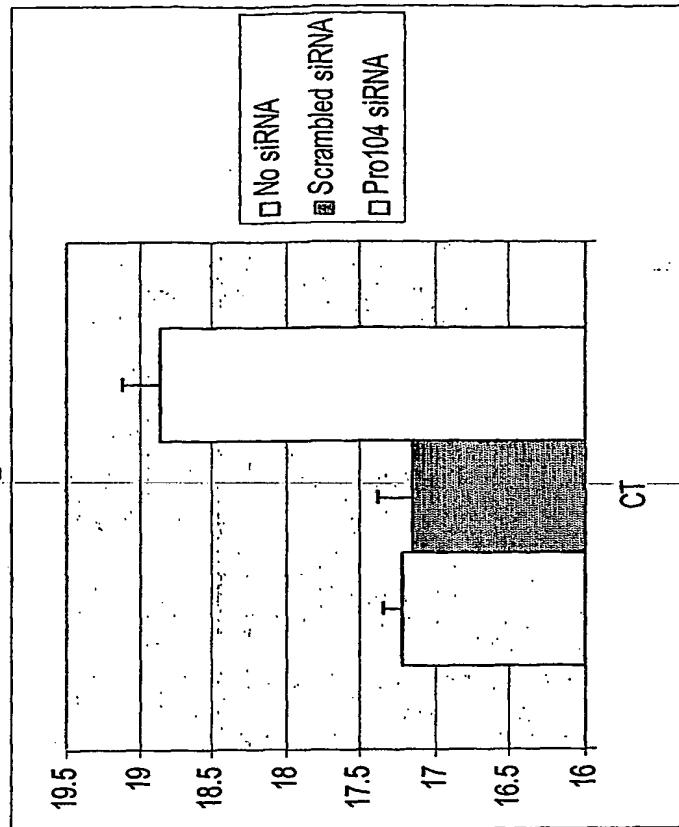


Fig. 24B



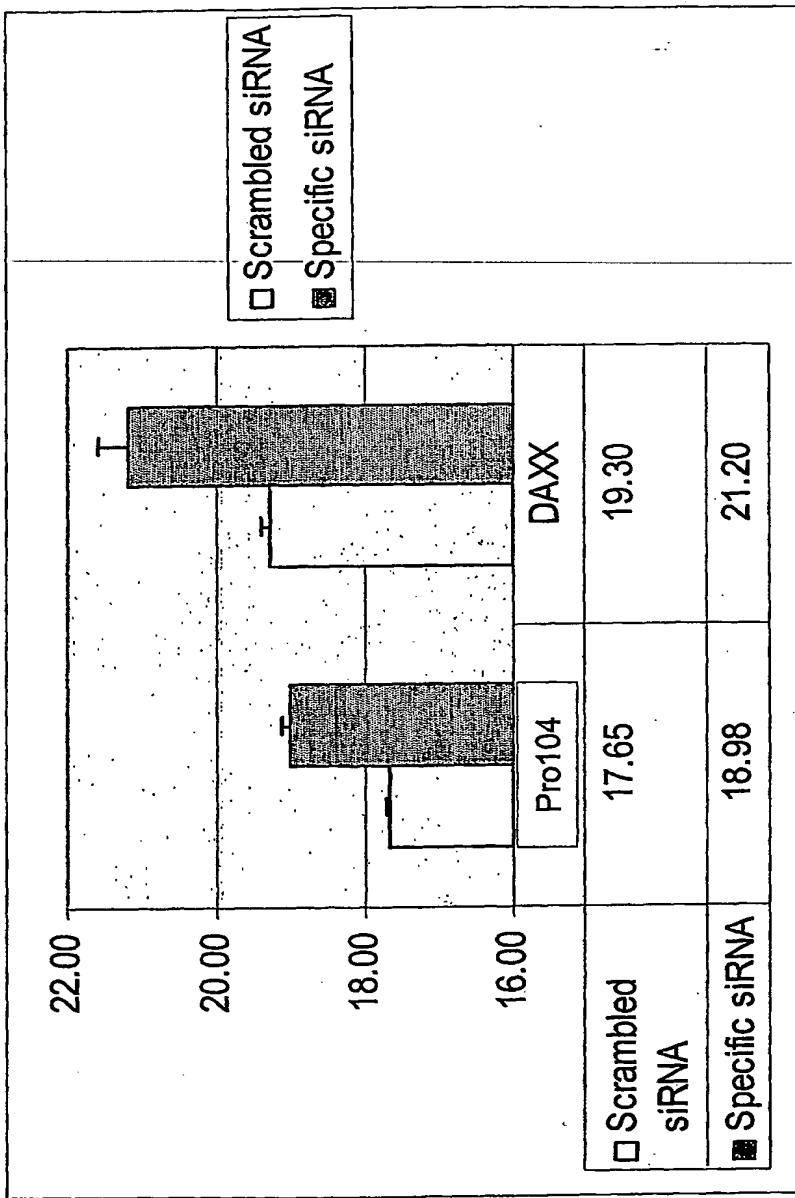
### GAPDH Q-PCR Primers

### Pro104 Q-PCR Primers

$\Delta CT = 2$  for Pro104 (Knockdown 75%)

## FIGURE 25

### Pro104 siRNA Specifically Knockdown Pro104 mRNA in HeLa Cells, Compared to a Positive Control



QPCR with specific primers for Pro104 and DAXX (positive control for apoptosis) demonstrates mRNA knockdown

# FIGURE 26

## Different Pro104 siRNAs Induce Specific mRNA Knockdown and Apoptosis in HeLa Cells

Fig. 26A

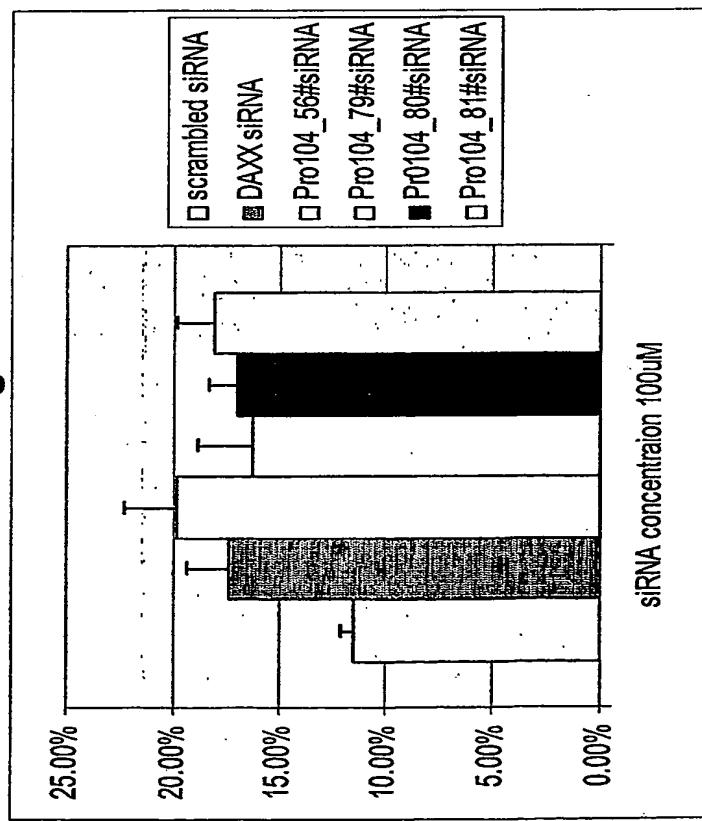
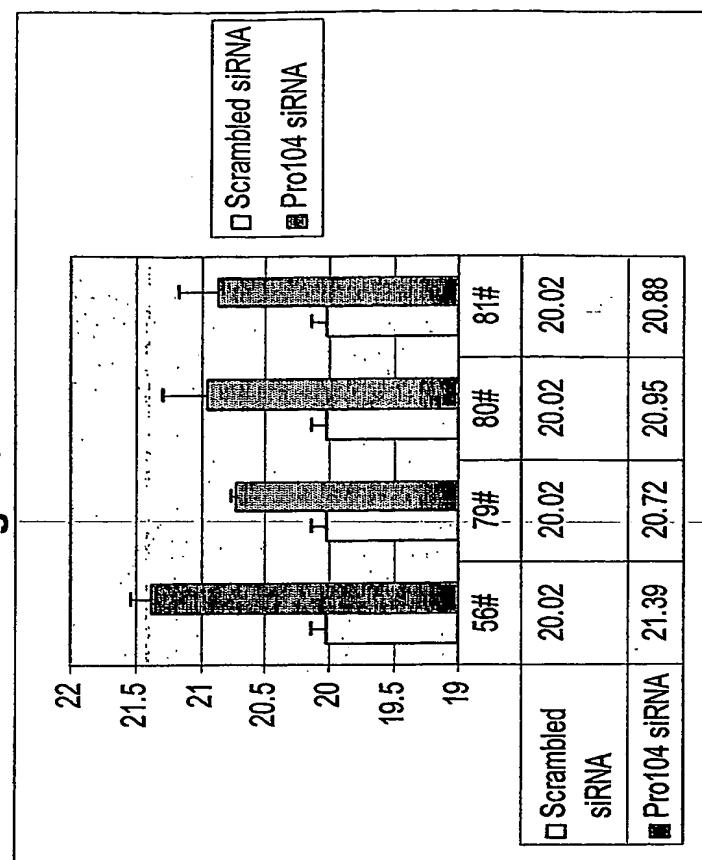


Fig. 26B



Annexin V assay for apoptosis  
(DAXX positive control)

QPCR demonstrates Pro104 mRNA  
knockdown

# FIGURE 27

## Specific Knockdown of Pro104 mRNA in HeLa Cells Induces Cell Death

Fig. 27A

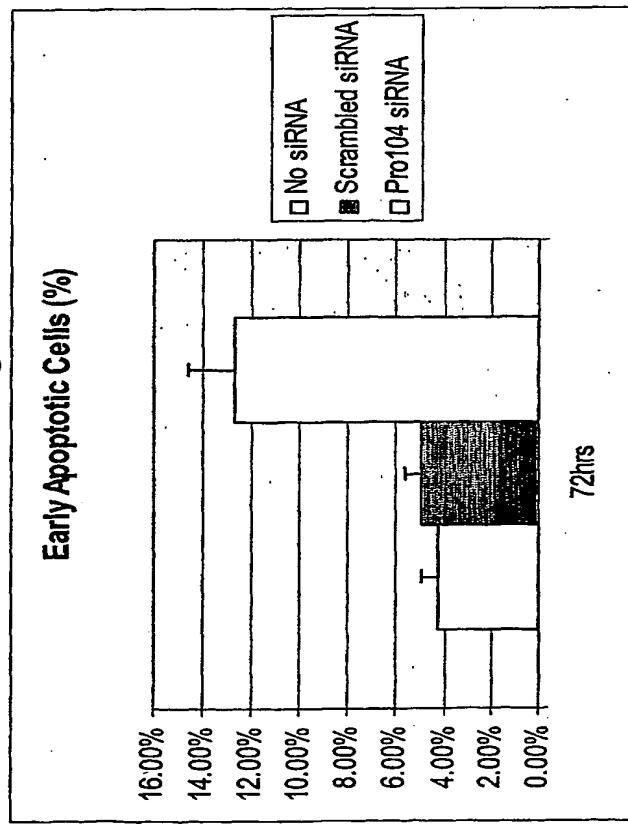
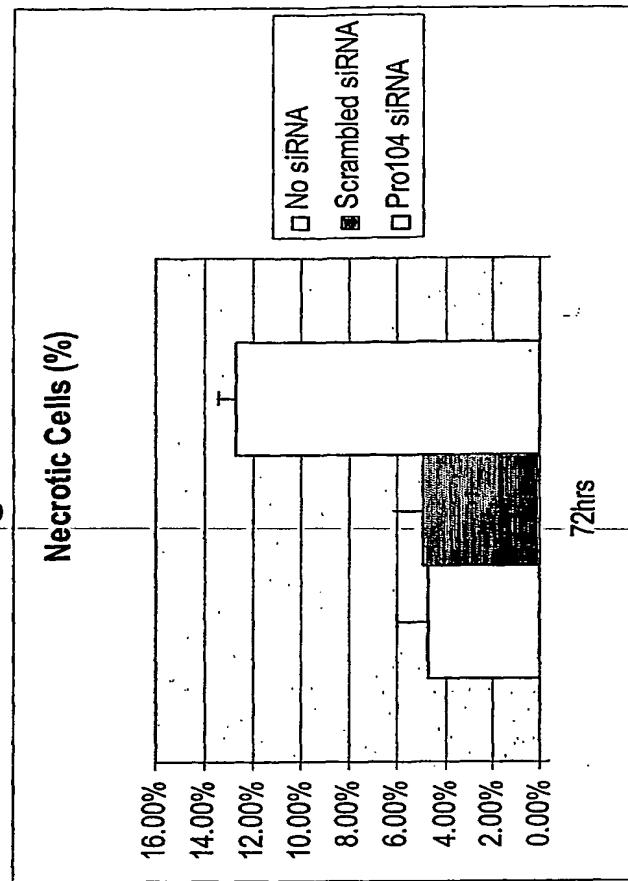


Fig. 27B



Annexin V Assay

## FIGURE 28

**Specific mRNA Knockdown by Pro104 siRNA  
Induces Apoptosis in HeLa Cells;  
Measured by Two Different Methods**

Fig. 28A

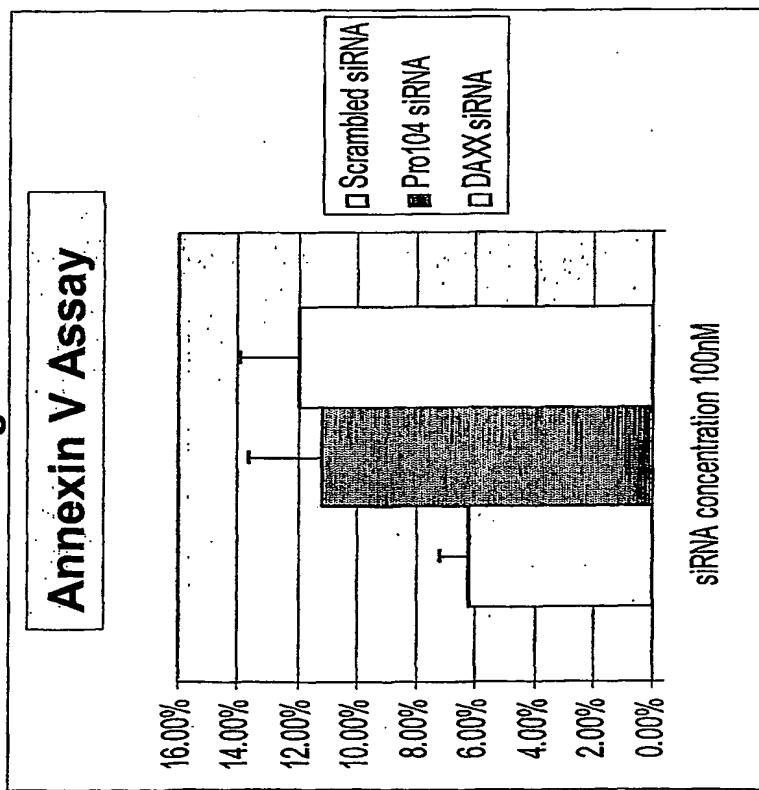
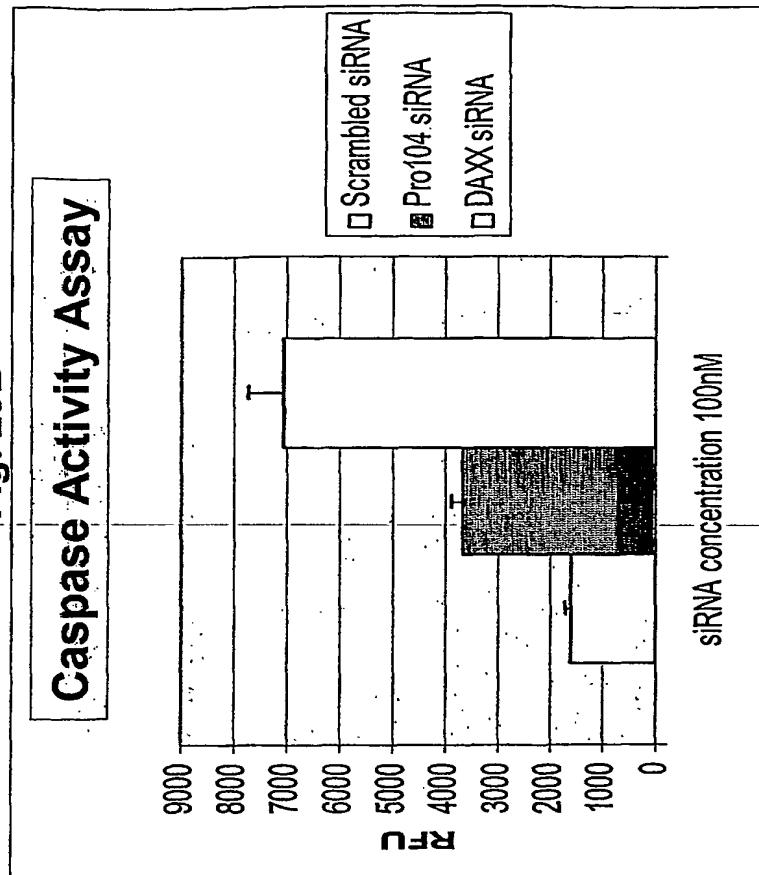


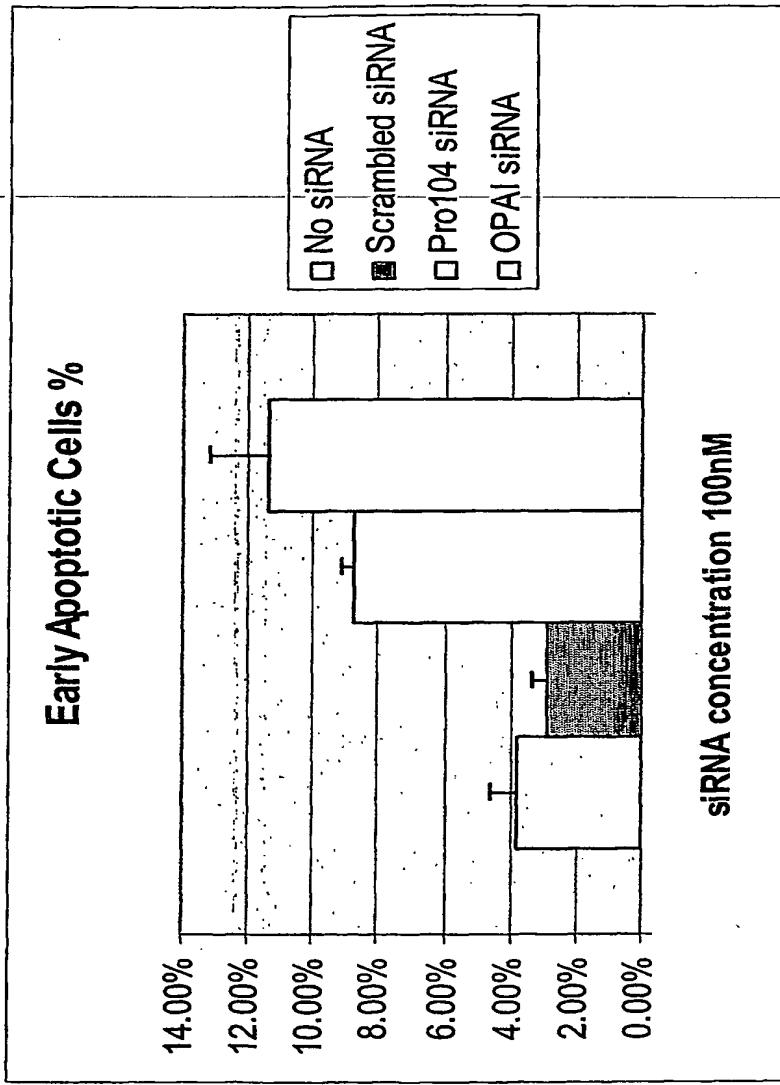
Fig. 29B



**Apoptosis assays of cells treated with Pro104 siRNA**

## FIGURE 29

### Specific Knockdown of Pro104 mRNA in CaOV3 Cells Induces Apoptosis



OPA1: Positive Control

Annexin V Assay

## FIGURE 30

### Pro104 siRNA Has no Effect on Apoptosis in Cells Without Pro104 mRNA

Fig. 30A

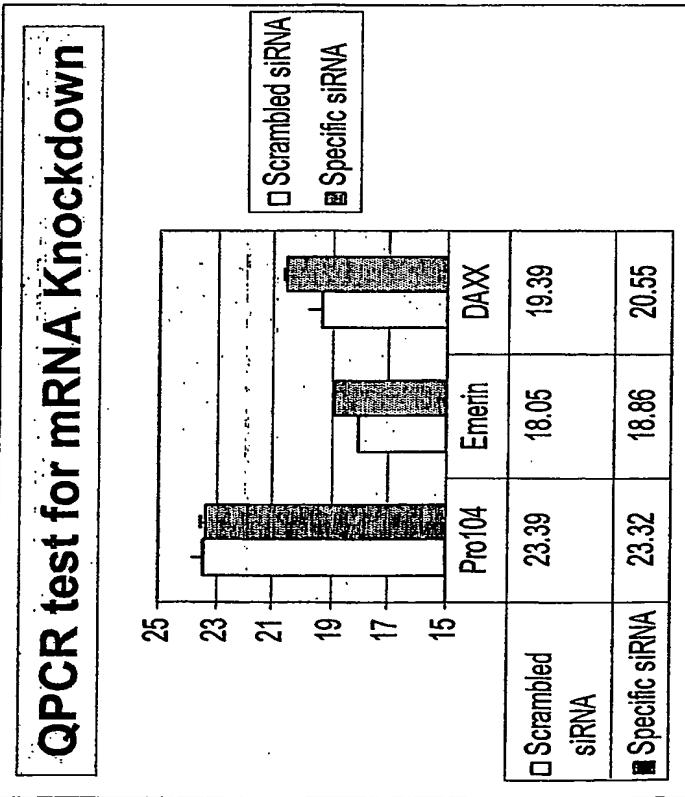
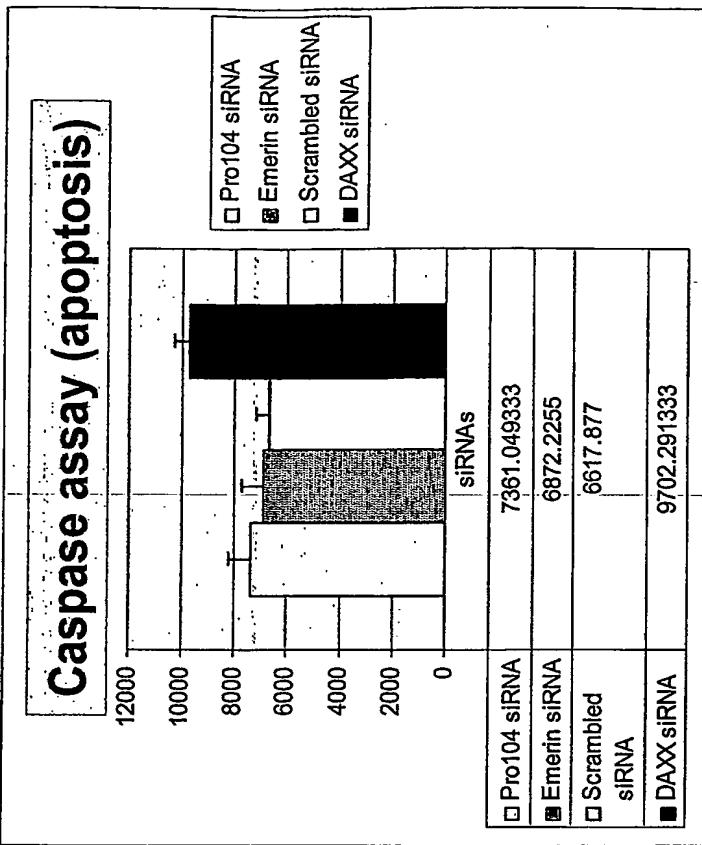


Fig. 30B



### SKBR3 cells

#### Knockdown:

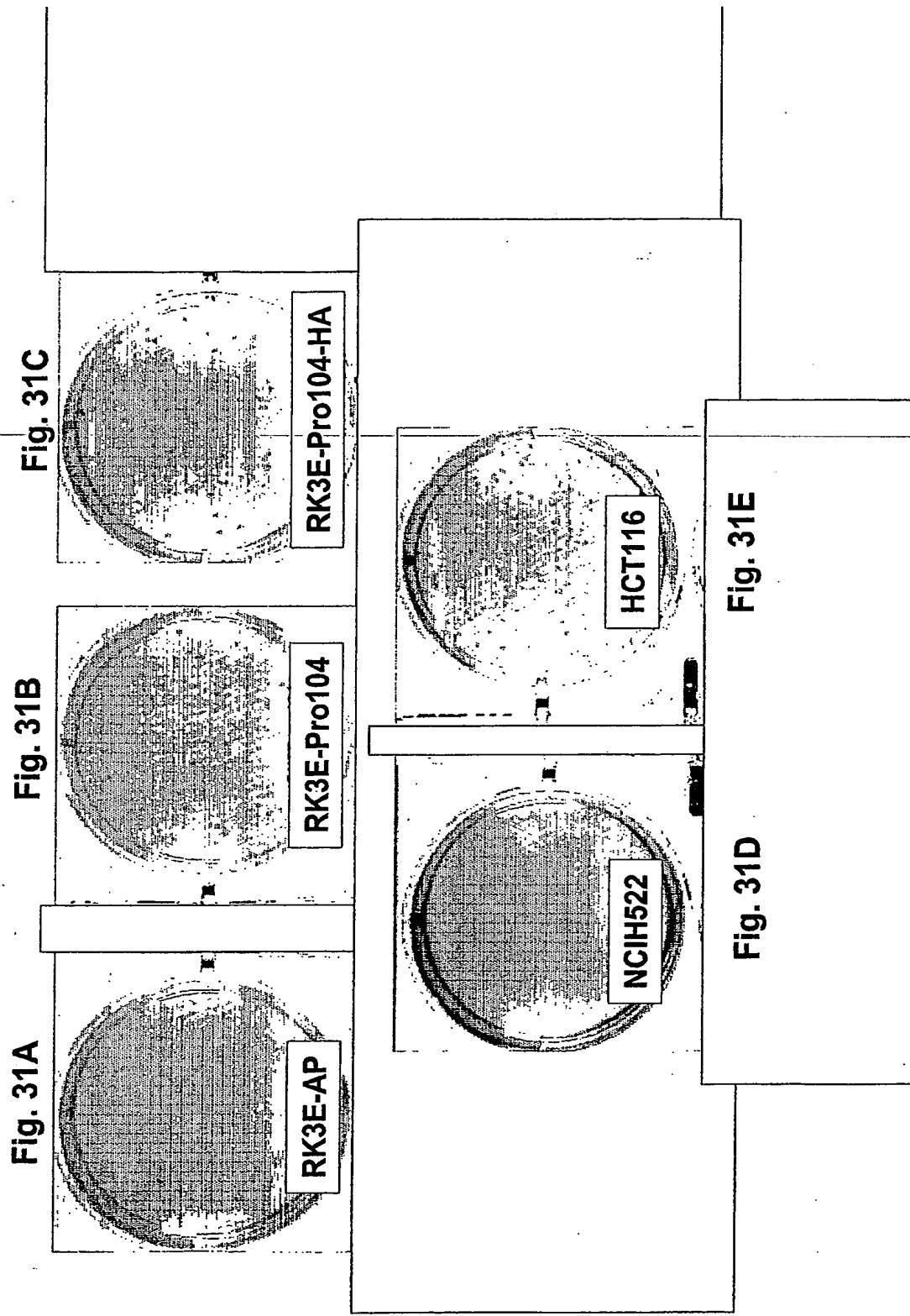
Pro104:none (no mRNA)

DAXX: 65% (positive control)

Emerin: 50% (mRNA +, non-essential)

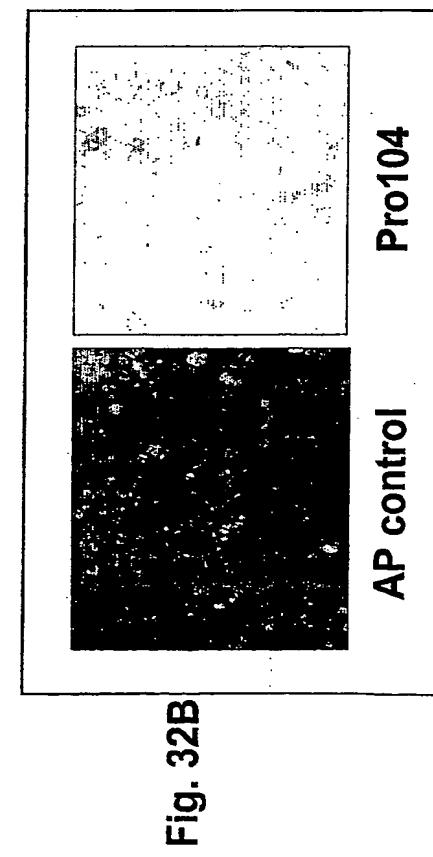
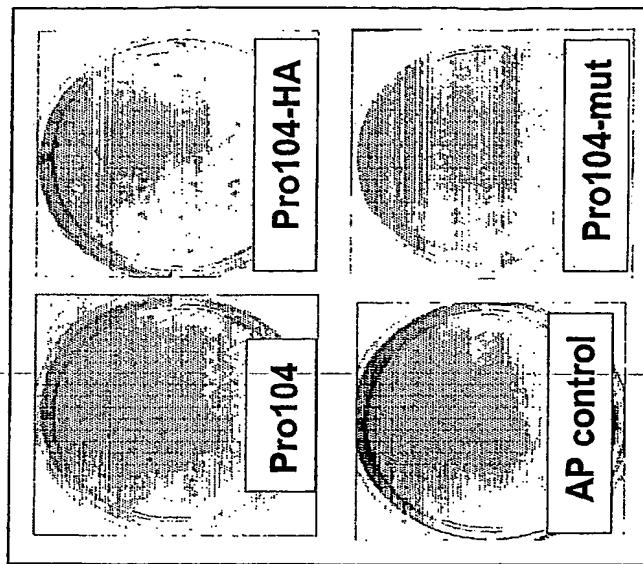
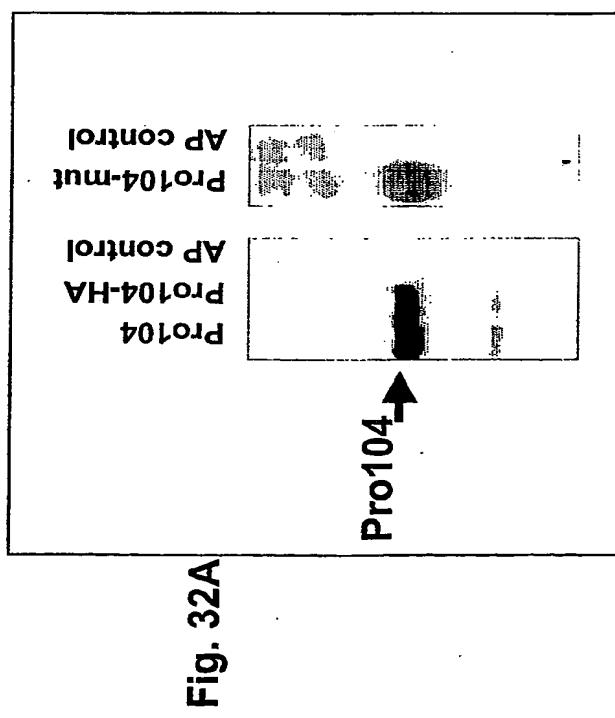
# FIGURE 31

## Overexpression of Pro104 Induces Cell Growth in Soft Agar



# FIGURE 32

## Pro104 Protease Activity is Required for Cell Growth



**Fig. 32D**

Cell Type	# Colonies per field
AP control	0
Pro104	60
Pro104-HA	68
Pro104-mut	0

RK3E cells with Pro104-mut lack Pro104 protease activity

# FIGURE 33

## Knockdown of Pro104 mRNA by siRNA Inhibits Growth of HeLa Cells in Soft Agar

Fig. 33A

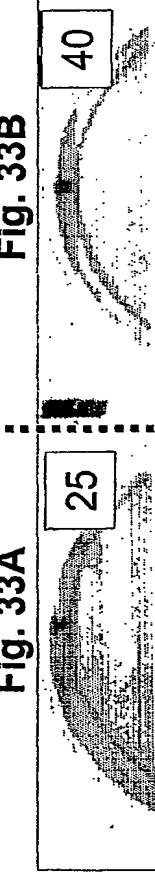


Fig. 33B

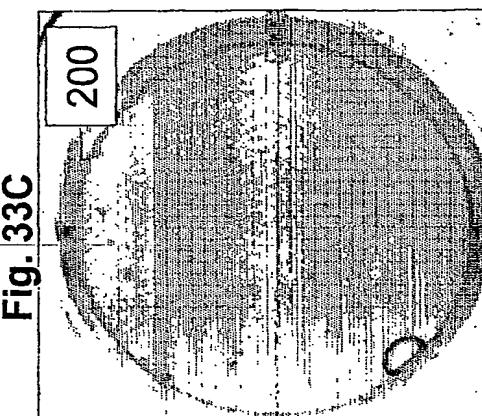
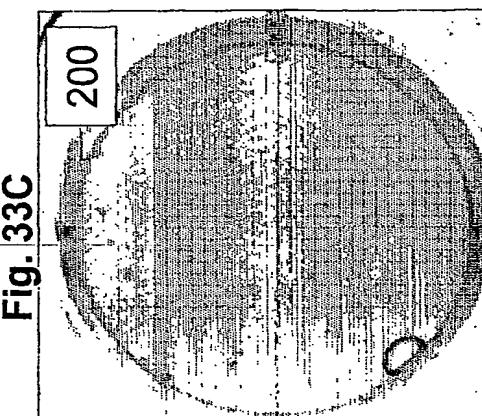


Fig. 33C



Pro104 siRNA

DAXX siRNA  
(apoptosis positive control)

Scrambled siRNA

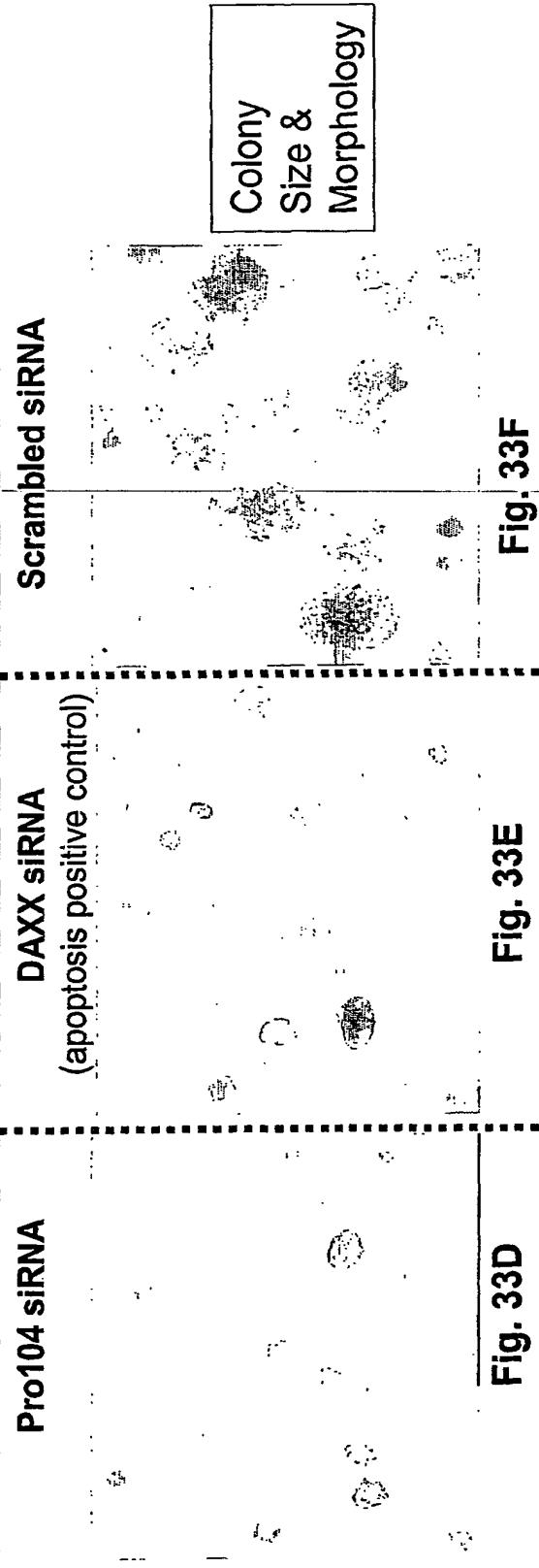


Fig. 33D

Fig. 33E

Fig. 33F

# FIGURE 34

## Knockdown of Pro104 mRNA by siRNA Inhibits Growth of HeLa Cells in Soft Agar

Fig. 34A

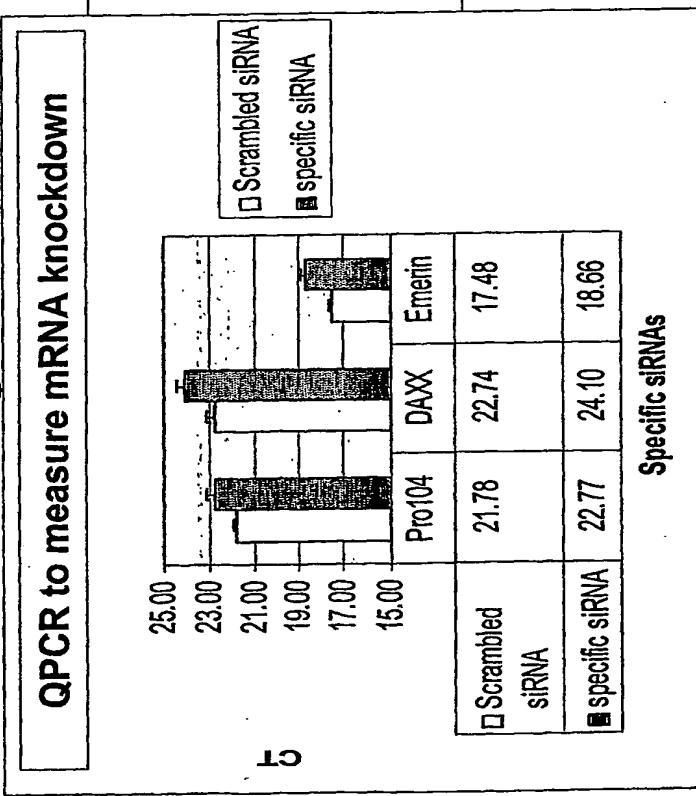
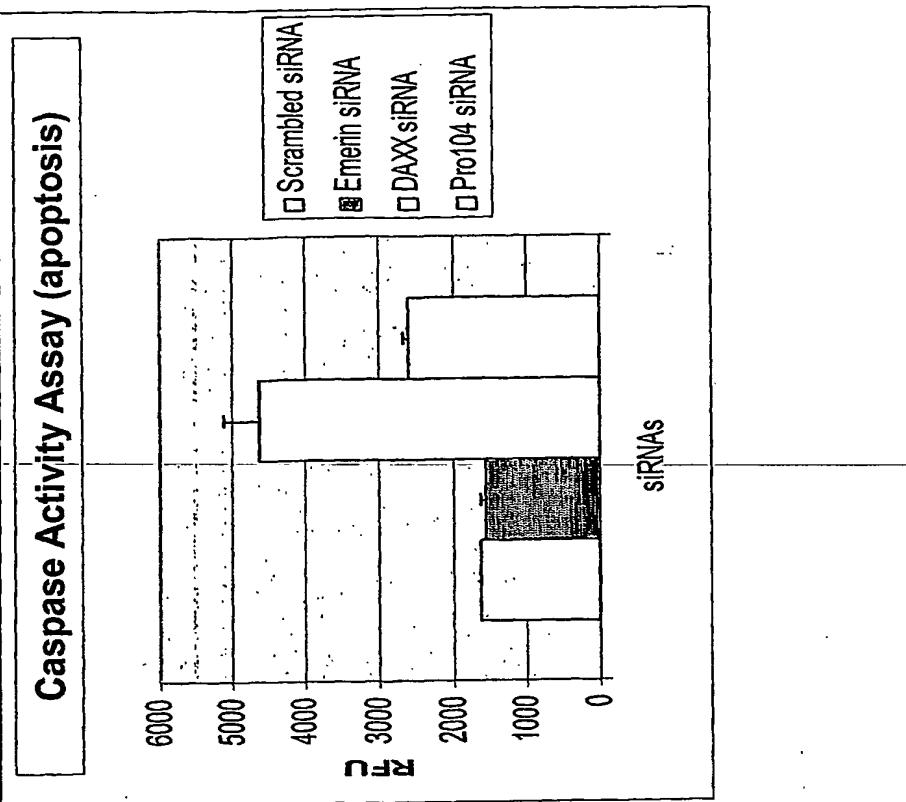


Fig. 34B



Knockdown:  
Pro104: 50%  
DAXX: 65%  
Emerin: 50%

# FIGURE 35

## Increased Growth of Human Tumor Cells Over-Expressing Pro104

Fig. 35A

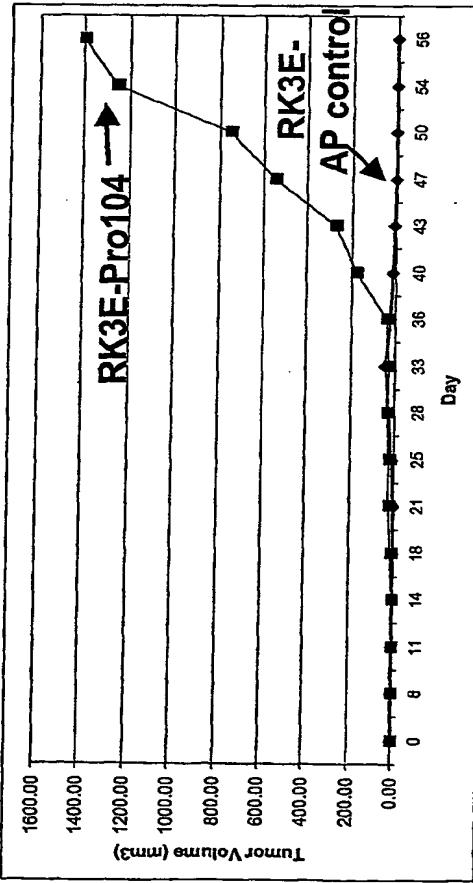


Fig. 35B

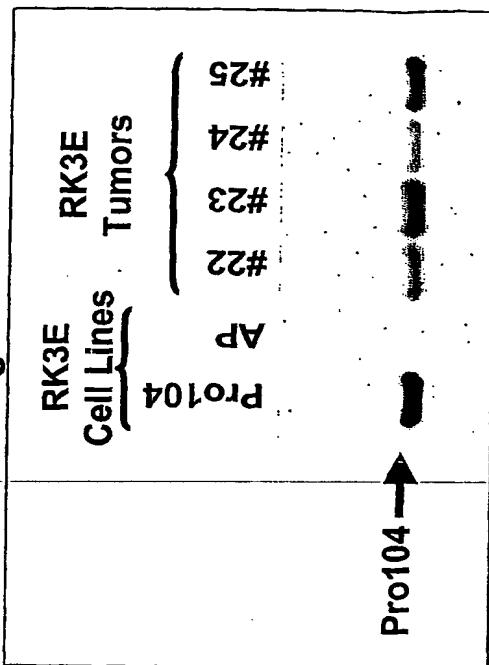
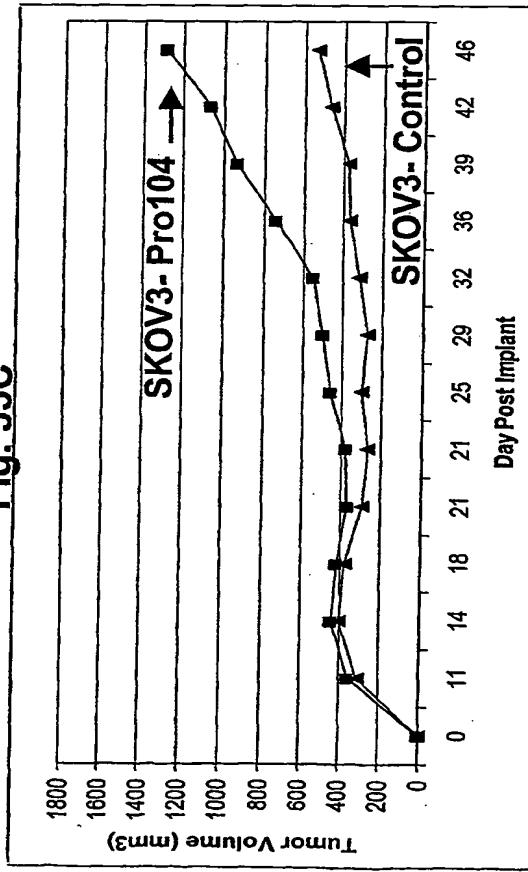


Fig. 35C



SKOV3 - Control

SKOV3 - Pro104



Pro104



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